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Oocyte—follicle cell dynamics in *Arrhenothrips ramakrishnae* Hood (Insecta: Thysanoptera)-I

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Abstract. Light and transmission electron microscopic studies of the developing oocytes from the germarium to the vitellogenic oocyte of the panoistic ovaries of *Arrhenothrips ramakrishnae* are included with a view to understand the sequential structural and behavioural changes involved in the development of the oocyte. Sufficient evidence is presented of these changes relating to the preliminary stages of development of the oocyte till the initiation of yolk formation and completion of the follicle cell epithelium, based on transmission electron microscopy.

Keywords. Germarium; oogonia; follicle epithelial cells; ooplasm; vitellarium; previtellogenic oocytes; yolk.

1. Introduction

The panoistic ovaries of *Arrhenothrips ramakrishnae* comprise 4 ovarioles on each side, with their apices independently joining with the terminal filament, a syncitial strand performing a suspensory function. In some species of thrips the ovariolar apices merge into the common germarium with a mass of oogonial cells with well stained nuclei occurring in all stages of development which join with the terminal filament (figure 1). Following observations on the gross structure of the ovaries of several thrips species (Sharga 1933) and of *A. ramakrishnae* (Ananthakrishnan 1955), information on the postembryonic development was provided by Davies (1961), Varadarasan and Ananthakrishnan (1981) and Ananthakrishnan (1984), confirming Heming's (1970) observations of the first indication of the approaching division of each ovary of the ovarian rudiment into 4 ovarioles in the prepupal stage of *Haplothrips verbasci*. While in the first pupal stage the 4 ovarioles are separated from each other, in the second they coalesce or join at their apices with the terminal filament. The rate of development of the ovarioles and individual oocytes in *Arrhenothrips* shows a distinct correlation with the preoviposition period of 8 days. There occurs a gradual development of individual ovarioles and oocytes from the second pupa to the second day of adult and a steep rise from the third day to oviposition (Varadarasan and Ananthakrishnan 1981). Of added significance is the occurrence of polymorphism with major and minor females and oedymerous and gynaecoid males besides normal males and females, so that it has become possible to analyse the mating patterns involved, the resulting fecundity and egg laying sequence. Haga (1985) has described the events taking place during oogenesis and embryogenesis in the Idolothripine species *Bactrothrips brevitubus*, while Dhileepan and Ananthakrishnan (1987) have discussed ovarian polymorphism in relation to reproductive diversity and associated histological and histochemical attributes in some sporophagous Tubuliferan Thysanoptera, also involving structural details of the developing oocytes based on light microscopic studies.

As information on the structural and behavioural changes of the developing oocytes as well as of follicle epithelial cells are aspects not known among thrips, an attempt has been made to highlight some of these aspects from the germarial cells to the mature oocyte, in particular the nature of development of the follicular epithelial cells vis-a-vis this oocyte, with the aid of light and transmission electron microscopy. A basic picture of the histology of the panoistic ovary and nature of vitellogenesis has been discussed (Bonhag 1958). The paper deals with the earlier stages of oocyte development, leaving the penultimate and mature oocytes for the second part.

2.   Materials and methods

The ovaries of *A. ramakrishnae* were dissected out in insect ringer solution and ovarian follicles at different stages of development were fixed in 2–3% gluteral-dehyde + 2–3% paraformaldehyde in phosphate buffer (pH 7·2) (0·1 M). After osmium tetroxide fixation, tissues were rinsed in buffer, keeping them in 1% uranyl acetate overnight at 0–4°C and then dehydrated. Eventually the material was embedded in an Epon-Araldite mixture and polymerized at 60°C and ultra thin
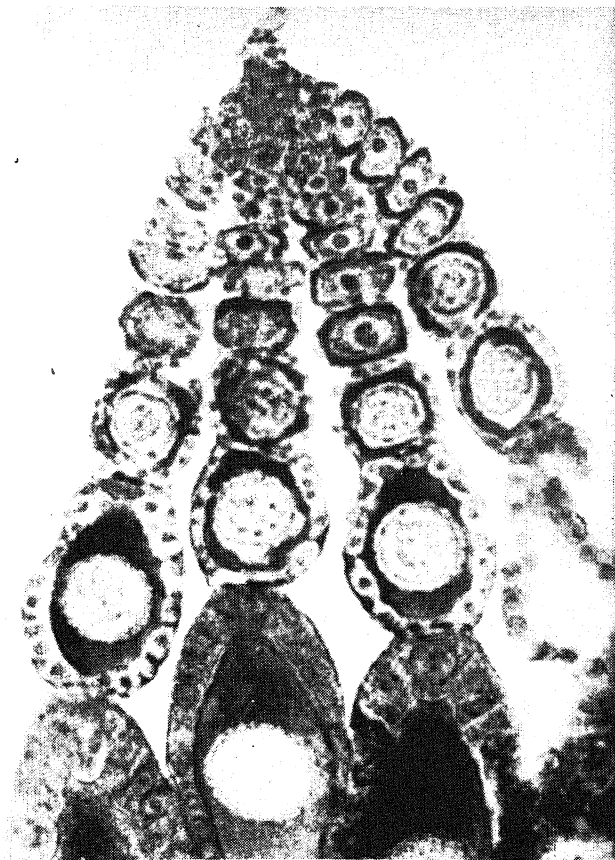
Figure 1. The panoistic ovary of *Haplothrips tardus* Priesner showing the fusion of the apical germarium region.

sections cut with an ultratome and examined in a transmission electron microscope (TEM).

3. Results

The germarium, the conical part of the ovariole apex comprises a mass of 10–15 small cells (figure 2A) with well stained nuclei in all stages of development is followed by the vitellarium, the largest part of the ovariole containing the developing oocytes (figures 2A–D and 3). The previtellogenic, vitellogenic and postvitellogenic zones are
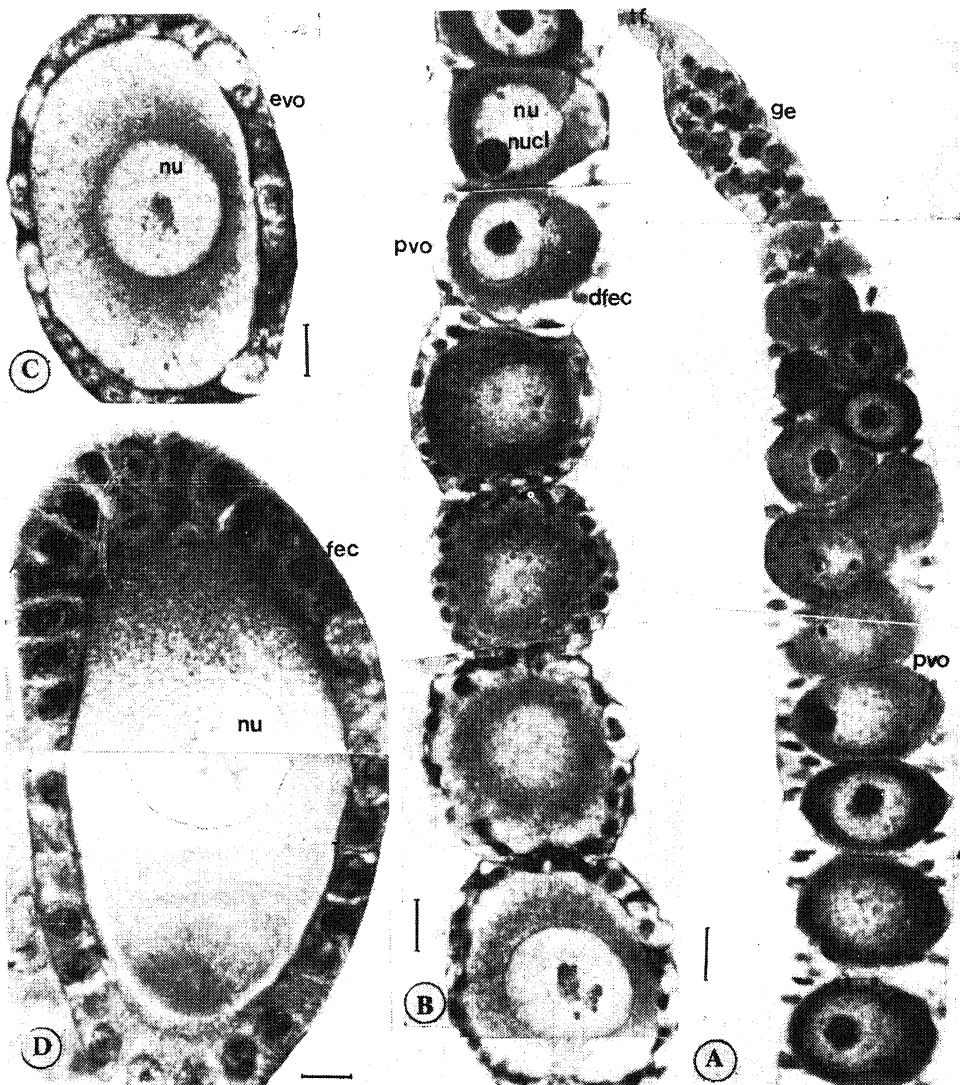
Figure 2. Light microscopic picture of the developing oocytes. **A.** Germarium with previtellogenic oocytes. **B.** Early vitellogenic oocytes with the formation of the follicular epithelial cells. **C.** Vitellogenic oocyte. **D.** Vitellogenic oocyte in late phase. (Bar = 10 μm).
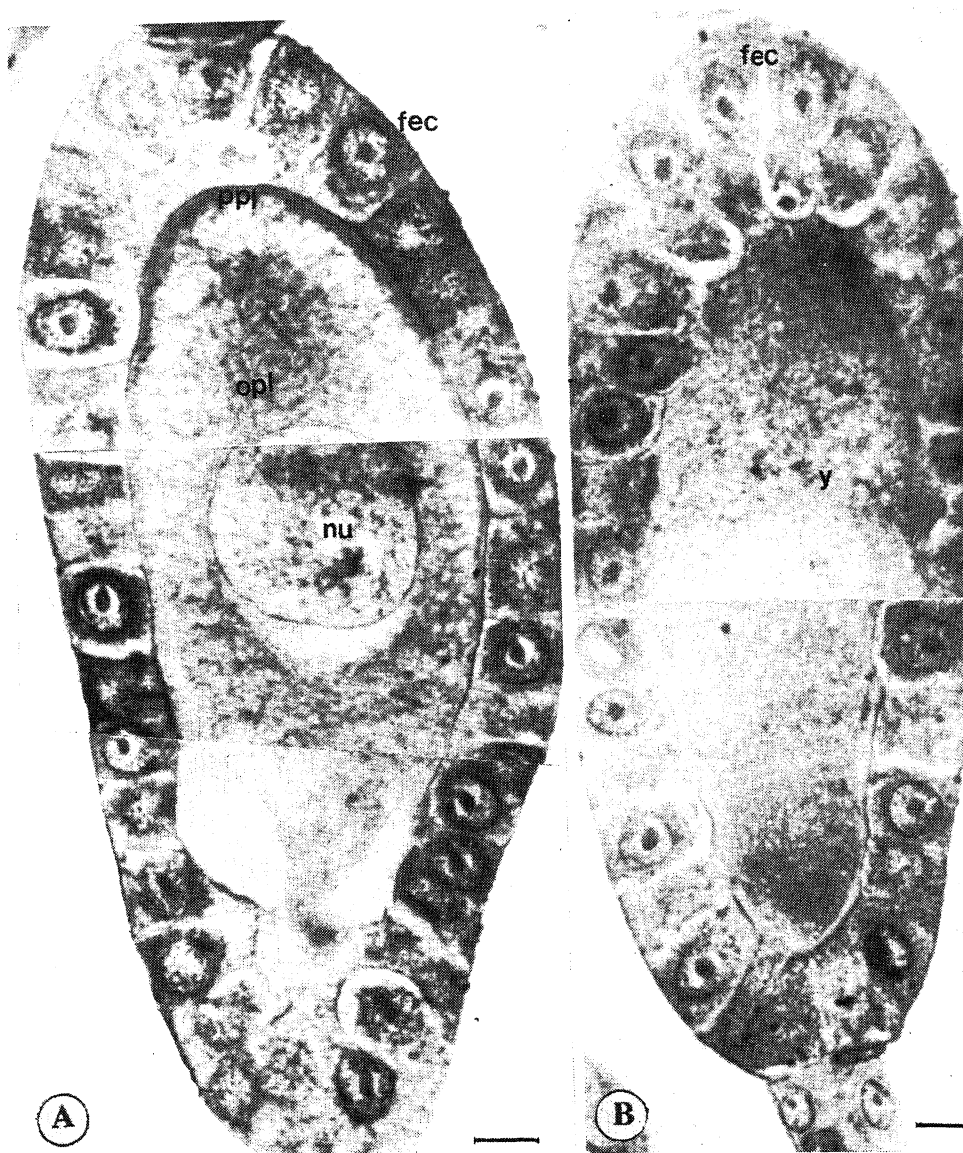
Figure 3. A and B. Penultimate and ultimate oocytes. (Bar = 10 μm).

clearly recognizable in the vitellarium. The number of mature oocytes in each ova
varies from 4–8, with 2–3 or occasionally 4–5 oocytes maturing in an ovary at a tir
    The germarium comprises the oogonial cells, each of which is a small spherical
with a distinct nucleus and peripheral cytoplasm and not completely enclosed by
pre-follicular cells (figure 6B). Each oogonium in the early stages is enveloped b
irregular indented plasma membrane (figure 6A). Various stages of developmen
the follicular epithelial cells are clearly discernable (figures 2A–D, 3, 4, 6C, D an
and with the development of the follicular epithelial cells, there appears to be
interlocking of the basal end of the follicular cell membrane with the oocyte plas
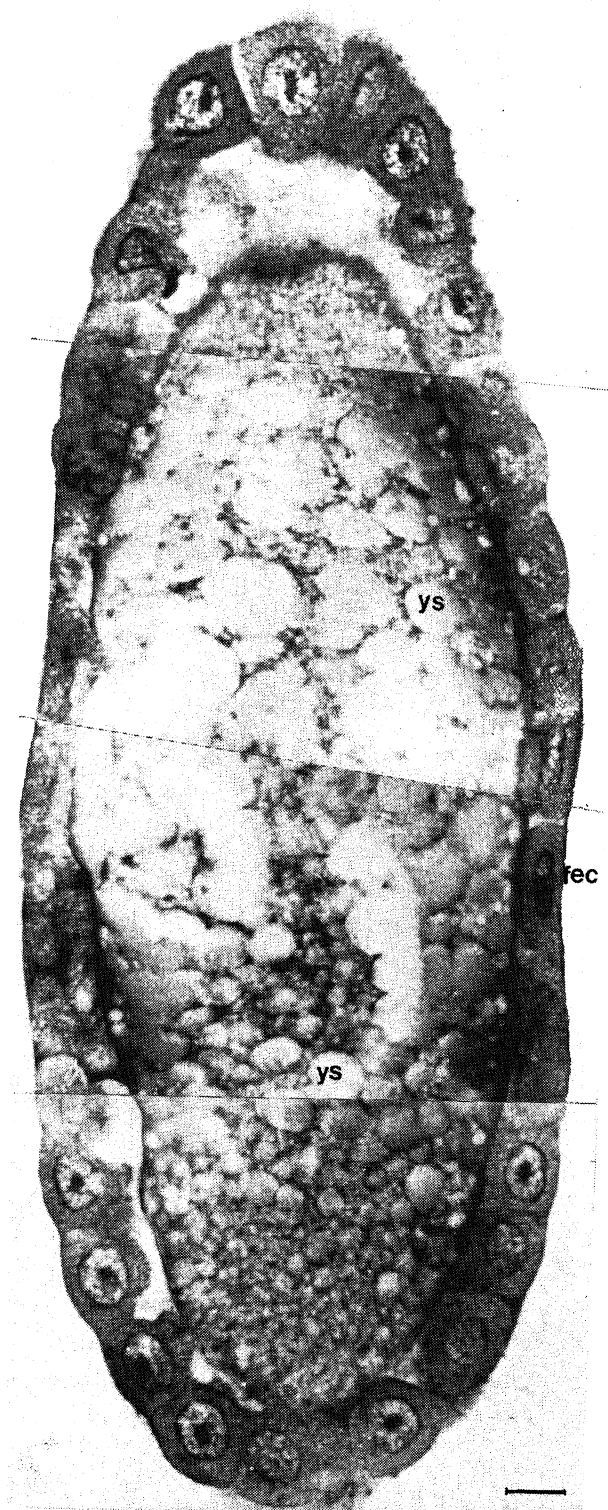
Figure 4. A mature oocyte showing the stretched follicular epithelial cells. (Bar = 10 μm).
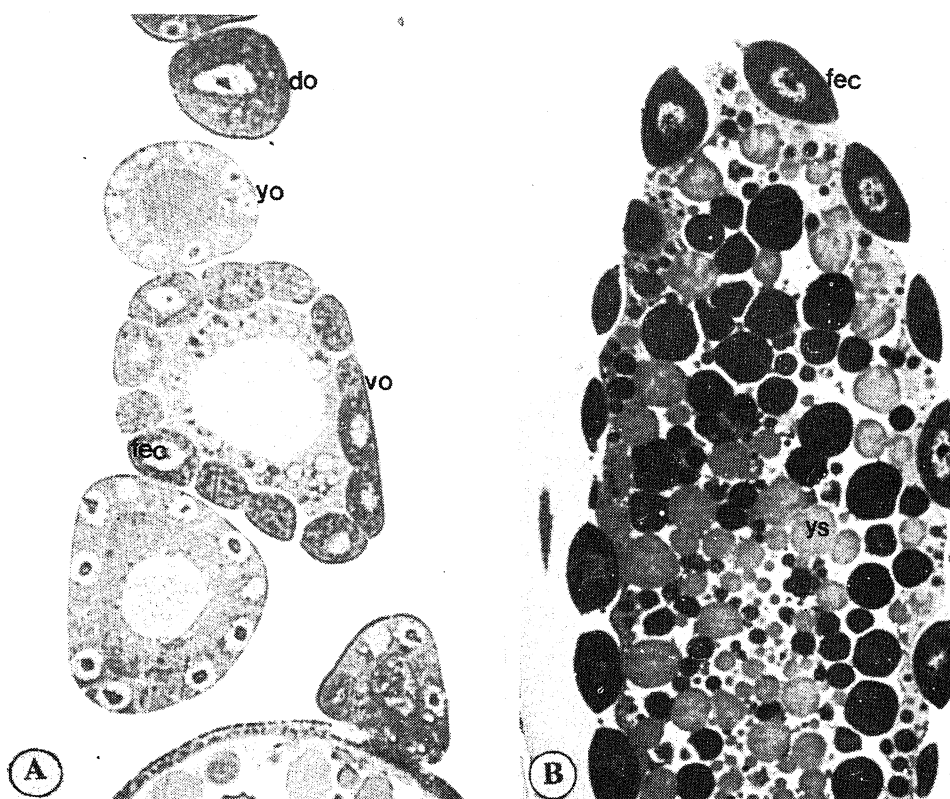
Figure 5. **A.** T.S of developing ovariole. **B.** L.S of mature oocyte.

membrane (figure 10) even in the initial stages of development. Further down, th developing oocytes are almost completely enclosed by a continuous layer of follic cells. As the oogonial cells descend down into the previtelline zone they appe spherical with a centrally placed nucleus, almost completely occupying most of th cell. The oocytes are arranged in a linear fashion surrounded by a thin layer follicular epithelial cells (figure 2B). The cortical ooplasm shows a sizable number vesicles of various types, during vitellogenesis, with some of them coated (figure Multivesicular bodies and lipid droplets as well as bodies with a tubular profile a also discernable (figure 8). There is also a tendency for a progressive fusion of th small vesicles into larger ones. This fusion appears to occur till the mature oocyt (figure 4) are filled up with yolk. All the 3 types of yolk, protein yolk, lipid yolk an carbohydrate yolk or glycogen granules are physically separate and easily recogni able through TEM pictures (figure 5B). While protein yolk has a limiting membran lipid yolk is seen as lipid droplets with no membrane (figure 5B).

4. Discussion

In all panoistic ovarioles the anterior portion of the germarium is composed oogonia adjacent to the terminal filament, the oogonia being succeeded by oocytes
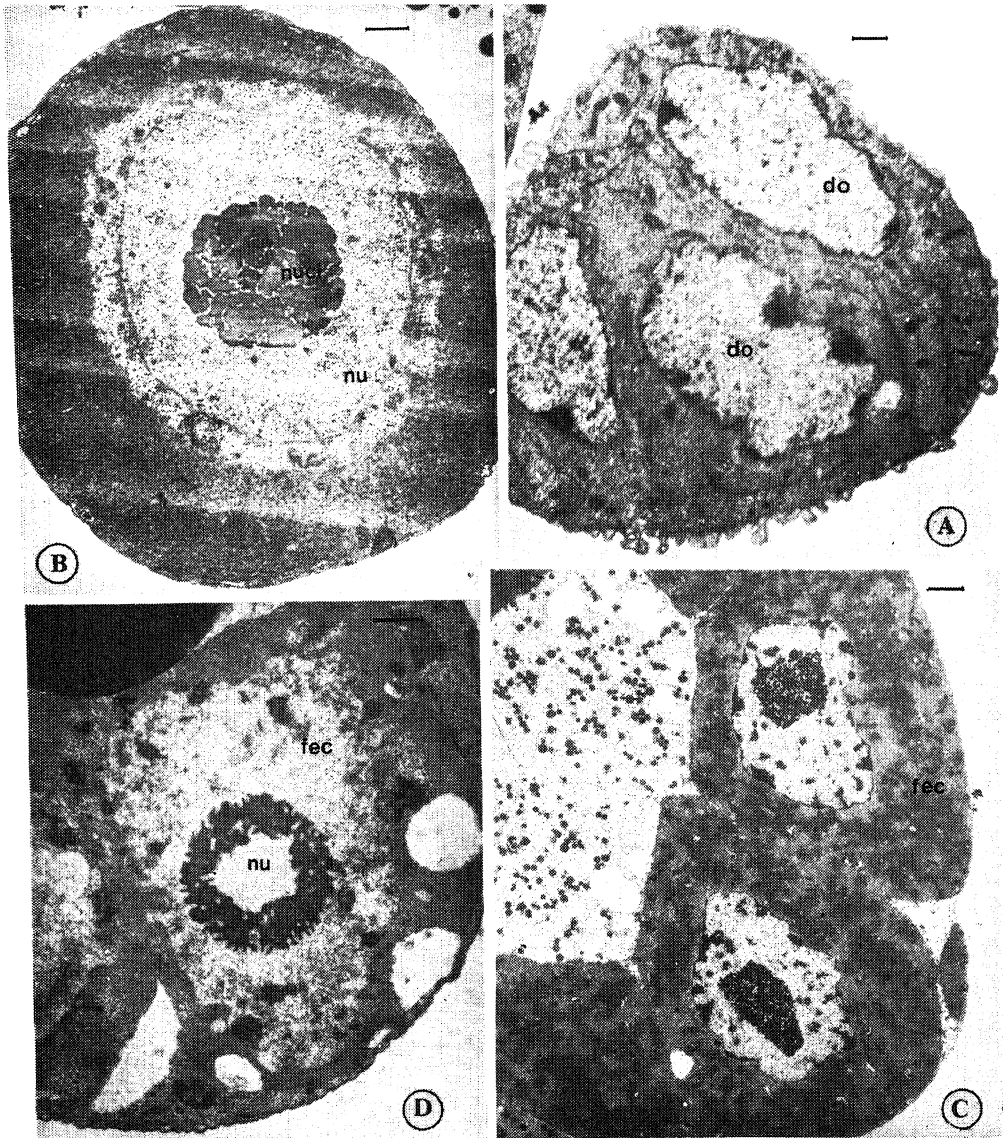
Figure 6. Early developmental sequence of oogonial and follicular cells. **A.** Oogonial cells in germarium. **B.** Primary oocyte with nucleus and nucleolus. **C.** Developing follicular epithelial cell. **D.** An enlarged view of follicular epithelium. (Bar = 10 μm).

the early stages of development and with the increase in cytoplasmic volume, the oocyte comes to occupy the whole width of the ovariole and becomes surrounded by the follicular epithelium. These aspects are confirmed through studies on the thrips ovarioles, wherein initially yolk appears across the follicular border as in other similar ovarian types, and subsequently fills the ooplasm. The follicular cells surround the growing oocyte during the early cytoplasmic growth phase and continue to divide until shortly before vitellogenesis (Mahowald 1972). Both light and transmis-
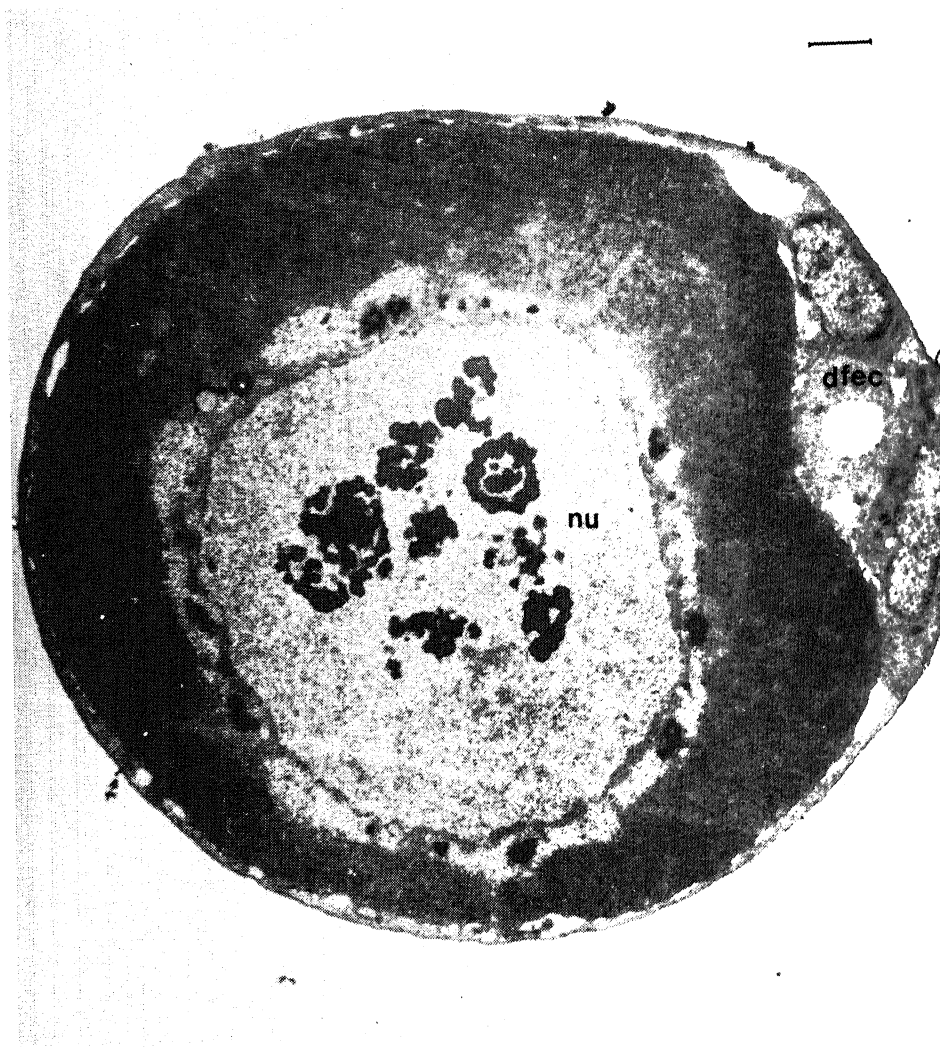
Figure 7. An early oocyte with follicular epithelial cell beginning to develop. (Bar = 10 µ

sion electron microscopic studies on the developmental and behavioural changes
the developing oocytes in *A. ramakrishnae* adequately emphasise the significance
the follicular cell epithelium and its relationship with the oocyte plasma membra
with which it appears to be very closely associated even from the early stages
development. Of equal interest is the mode of development and structural co
plexities of the germarial cells, which when they become transformed into the p
vitellogenic oocytes show clearly the various stages of differentiation of follicu
epithelial cells, the morphology of which has led to the presumption that they gov
the transmission of yolk precursors from the blood (Telfer 1965). The differentiati
of the oocyte cortex and the presence of coated pits and vesicles appear similar
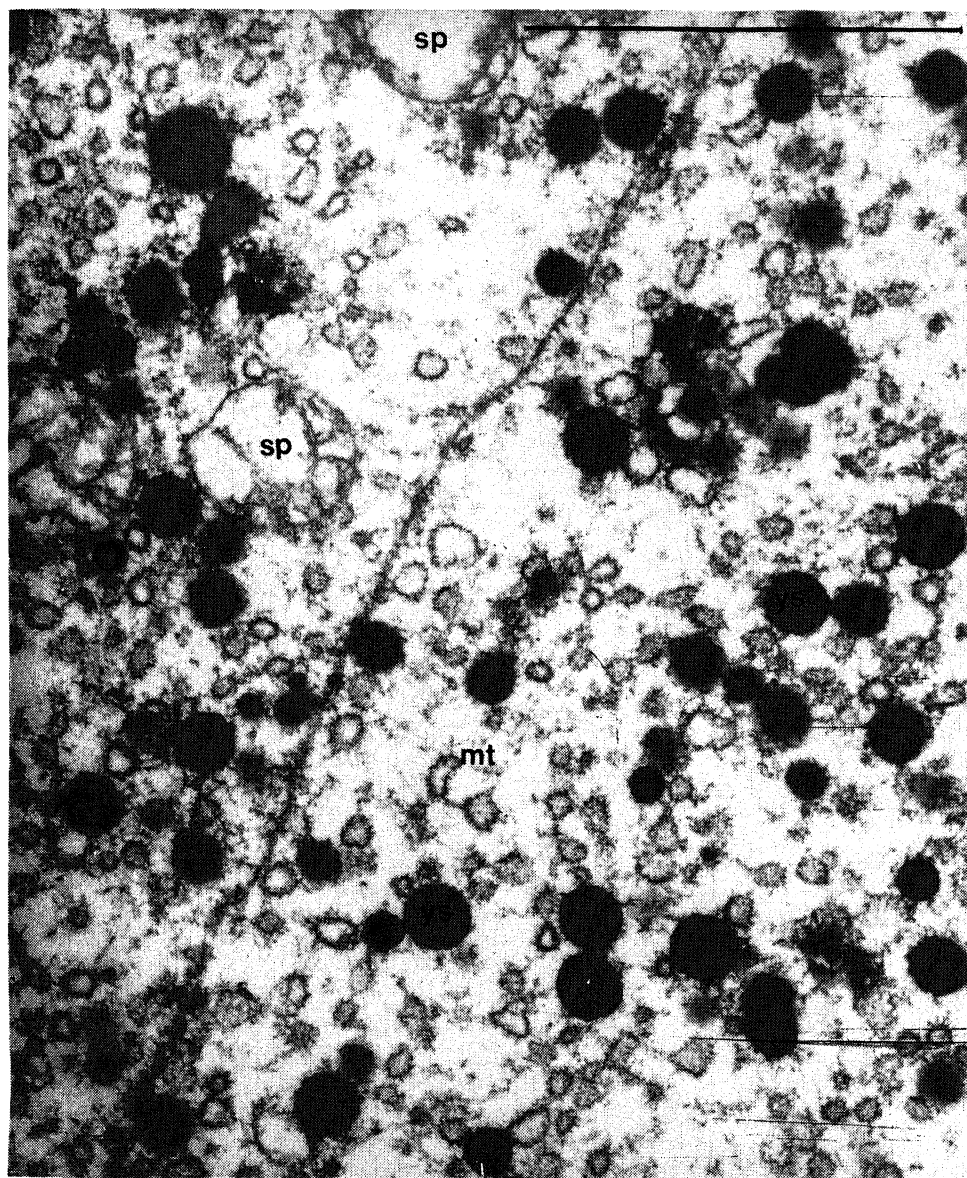
Figure 8. Immature follicle and oocyte cells showing dense granules in association with rough ergastoplasm and some microtubules. (Bar = 10 μm).

that of other insects, like *Bacillus rossius* (Rossi) with panoistic ovaries (Giorgi and Mazzini 1984), besides developing microvilli of the oocyte surface which interlock with similar projections arising from the basal ends of the adjacent follicular cells. Microvillar development and mitochondrial accumulation evident in many insects (Goltzene 1979) as well as the numerous types of vesicles present in the cortical ooplasm and the interior oocytes are equally typical of the developing oocytes of thrips.
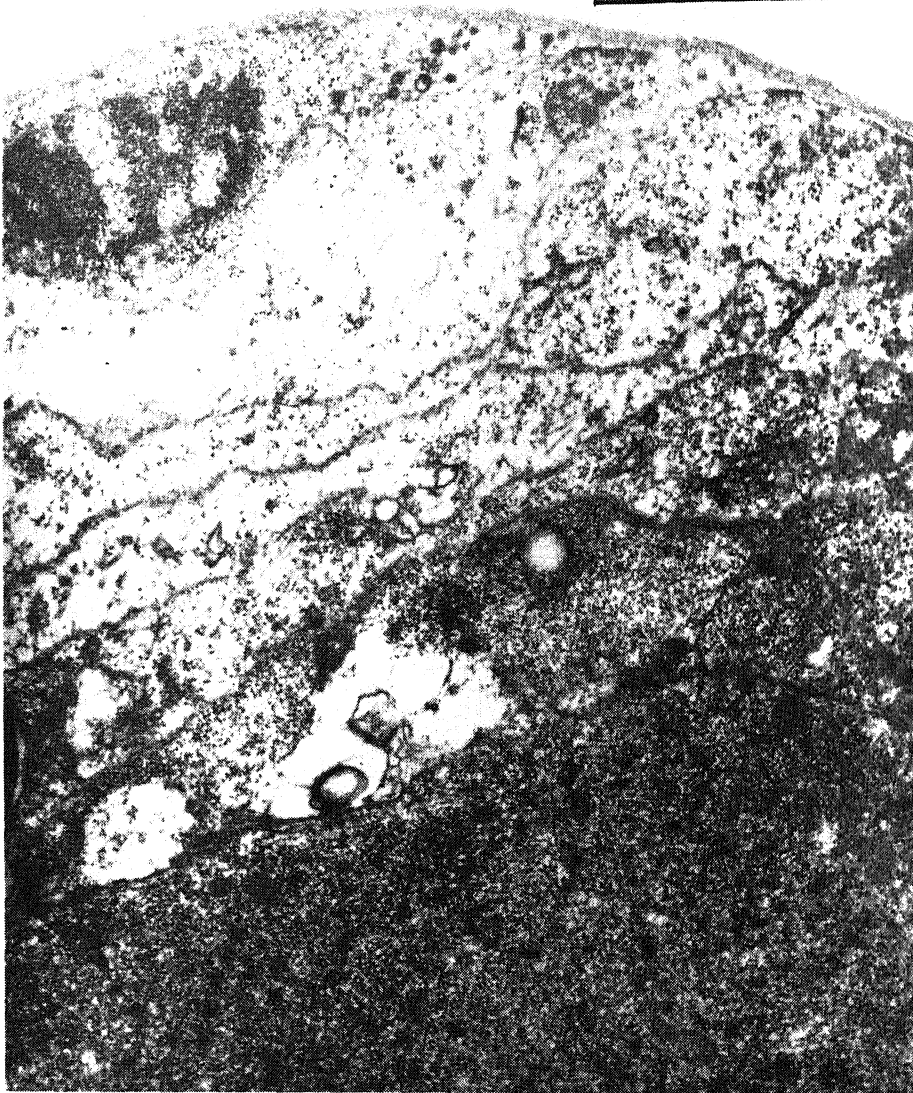
Figure 9. Surface of an immature oocyte showing very dense granules and with
follicular epithelial cells. (Bar = 10 μm).

Acknowledgements
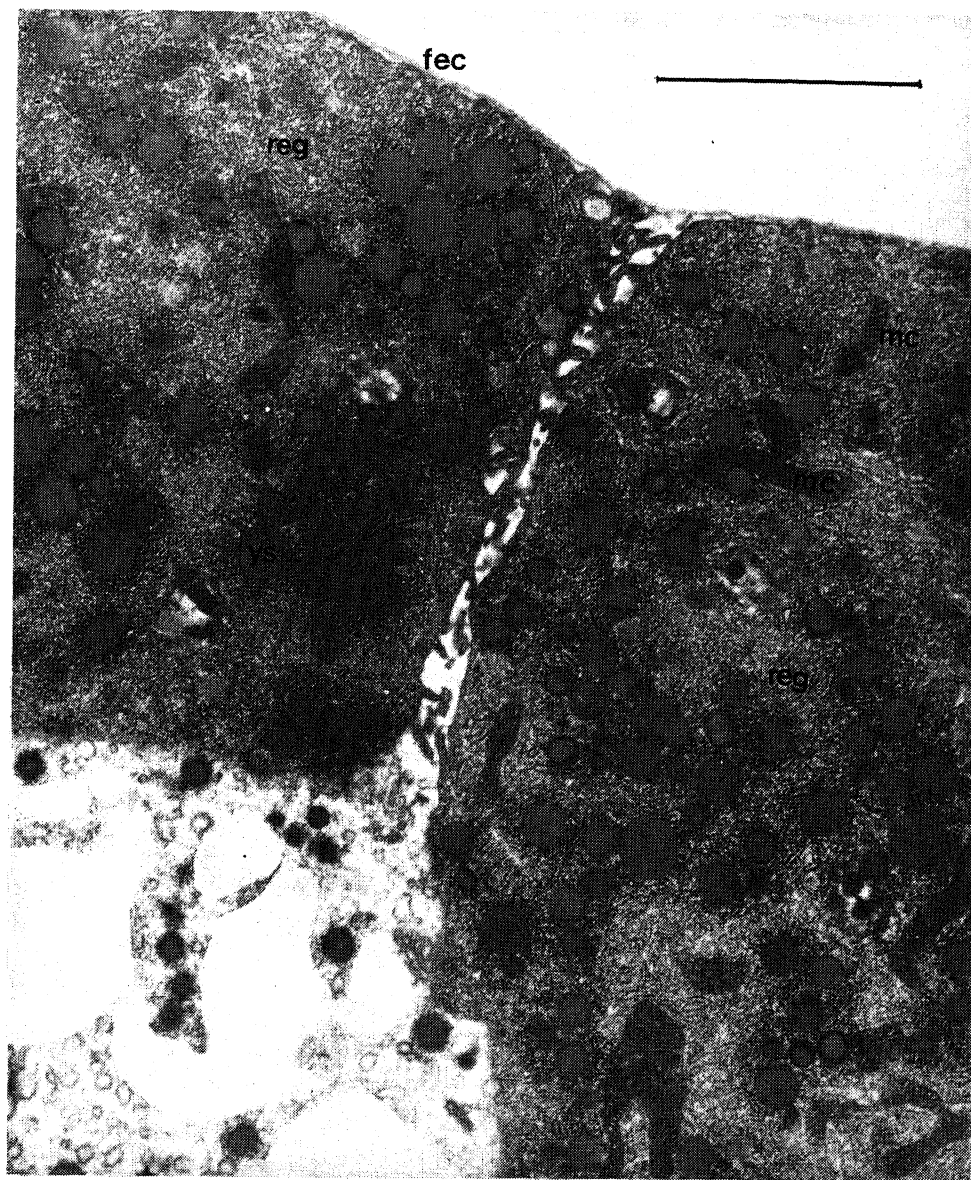
Figure 10. Early differentiating follicular cell with elongated mitochondria and rough ergastoplasm. Note villi like inter connections between follicular cells. (Bar = 10 μm).

(Abbreviations used: dfec, Developing follicular epithelial cell; do, developing oocyte; evo, early vitellogenic oocyte; fec, follicular epithelial cell; ge, germarium; mc, mitochondria; mt, microtubule; nu, nucleus; nucl, nucleolus; opl, ooplasm; ppl, periplasm; Pvo, pre-vitellogenic oocyte; reg, rough ergastoplasm; sp, sphere; tf, terminal filament; y, yolk; yo, young oocyte; ys, yolk sphere; vo, vitellogenic oocyte).

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Oocyte—follicle epithelial cell dynamics in *Arrhenothrips ramakrishnae* Hood (Insecta: Thysanoptera)—II. Observations on the preterminal and terminal oocytes

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Abstract. Transmission electron microscopic studies relating to the changes taking place in the follicular epithelial cells and oocyte cytoplasm of the penultimate and terminal oocytes of *Arrhenothrips ramakrishnae* Hood during vitellogenesis and the role of the follicular cells in this process are discussed.

1. Introduction

The sequential structural and behavioural changes involved in the preliminary stages of oocyte development in *Arrhenothrips ramakrishnae*, involving differentiation of the oocytes, development of the follicular epithelial cells and initiation of yolk formation on the basis of both light microscopic and ultrastructure studies through the transmission electron microscopy (TEM) were described by Ananthakrishnan (1988). The previtellogenic and vitellogenic oocytes indicated the presence in their ooplasm of diverse vesicular bodies and lipid droplets. Many of the yolk spheres in insect eggs appear to be formed from extracellular protein takeup at the oocyte surface by pinocytosis (Telfer 1965). Both the ovarian wall and the follicular epithelial cells provide sufficient evidence of this presumptive function regarding the transfer of yolk precursors from the blood, involving and endocytic sequestration from the haemolymph (Telfer *et al* 1982; Mazzini *et al* 1986). Besides, the oocytes also appear to have the capability of forming yolk by autosynthesis or synthesizing their own store of yolk. The differentiation of the follicular epithelium and the oocyte also appears to be a temporally coordinated developmental process, since the follicle-oocyte interaction is vital for vitellogenesis. While the penultimate oocyte is in the early vitellogenic phase, the mature oocyte is vitellogenic and consequently is both structurally and functionally different. Having indicated through TEM studies the mode of development of the follicle epithelial cells (Ananthakrishnan 1988) and the intimacy of contact between them and the oocytes, an attempt has been made to discuss the ensuing changes in the penultimate and mature oocytes which would throw sufficient light on their functional role.

2. Material and methods

The ovaries of *A. ramakrishnae* were dissected out in insect ringer solution and ovarian follicles at different stages of development were fixed in 2–3% gluteraldehyde + 2–3% paraformaldehyde in phosphate buffer (pH 7·2) (0·1 M). After osmium

tetroxide fixation, tissues were rinsed in buffer, keeping them in 1% uranyl acetate overnight at 0–4°C and then dehydrated. Eventually the material was embedded in an Epen-araldite mixture and polymerised at 60°C and ultra thin section cut with an ultratome and examined in a TEM.

Spurr's plastic embedded sections (0·5 μ) stained with Mallory's methylene blue-Axure II mixture were used for examination of the yolk spheres.

3. Results

The preterminal oocyte like its predecessors is enveloped by a single layer of follicular cells with prominent nuclei and nucleoli. The cytoplasm of the penultimate oocyte stains deeply and contains, besides many mitochondria, several vesicular bodies. The rough nature of the endoplasmic reticulum is not very evident and the numerous vesicles and tubular elements observed may represent the early phases of break down products involving organelles such as the mitochondria. The appearance of yolk spheres, and vesicles marks the initiation of vitellogenesis which differs from the more rapid process in the mature oocyte. The increased presence of the degradation products of the organelles as indicated by the vesicular bodies implies a reduced functional involvement in endocytic uptake, an aspect wall developed in the terminal oocyte. Though particular yolk granules are absent in the mature oocyte, refractile particles in the cytoplasm are observable when viewed with dark field optics. Smaller yolk granules are also found dispersed among the larger yolk spheres which are most often surrounded by a membrane (figures 1 and 2). Besides the protein and lipid yolk, carbohydrate yolk or glycogen also tends to accumulate towards the end of vitellogenesis (figure 5A). Much of the cytoplasm appears to be restricted to the cortex of the mature oocyte in contrast to the immature oocytes which are filled with deeply stained cytoplasm. Depending upon the developmental stage of the oocyte, the cytoplasm also contains from a few to several mitochondria. However cellular organelles are somewhat restricted to the periphery of the mature oocyte which is almost in close contact with the outer membrane, which while appearing as a continuous layer nevertheless shows in some thin sections pore-like structures, that may establish continuity between the intracellular and extracellular spaces (figure 3). In the earlier stages of the development of the oocyte it was observed that deposition of yolk commences with the close interlocking of the microvilli originating from the cortical ooplasm with those of follicular cells. As a result of rapid vitellogenic growth of the mature oocyte, the follicle cell-oocyte interface tends to get progressively widened, leading to the disappearance of microvilli (figure 4). There is also accumulation of a number of large vesicles in the cortical ooplasm, some of them membrane bound (figures 5A and 7). As a result of sustained pinocytosis, some of the cytoplasmic components become displaced so as to concentrate below the oocyte cortex in an area particularly rich in mitochondria (figure 8).

Externally the mature oocyte is covered by a single layer of follicular cells which have a prominent nucleus and nucleolus. In both the immature and mature oocytes the cytoplasm of the follicle cells stains deeply and is granular. Their dense staining could be attributed to the numerous electron opaque vesicles in the cytoplasm when stained with uranyl acetate and lead citrate. These spherical vesicles visible in cross section of the oocytes fill the cytoplasm of the mature follicle cell and occur in close association with rough endoplasmic reticulum, associated with which are the
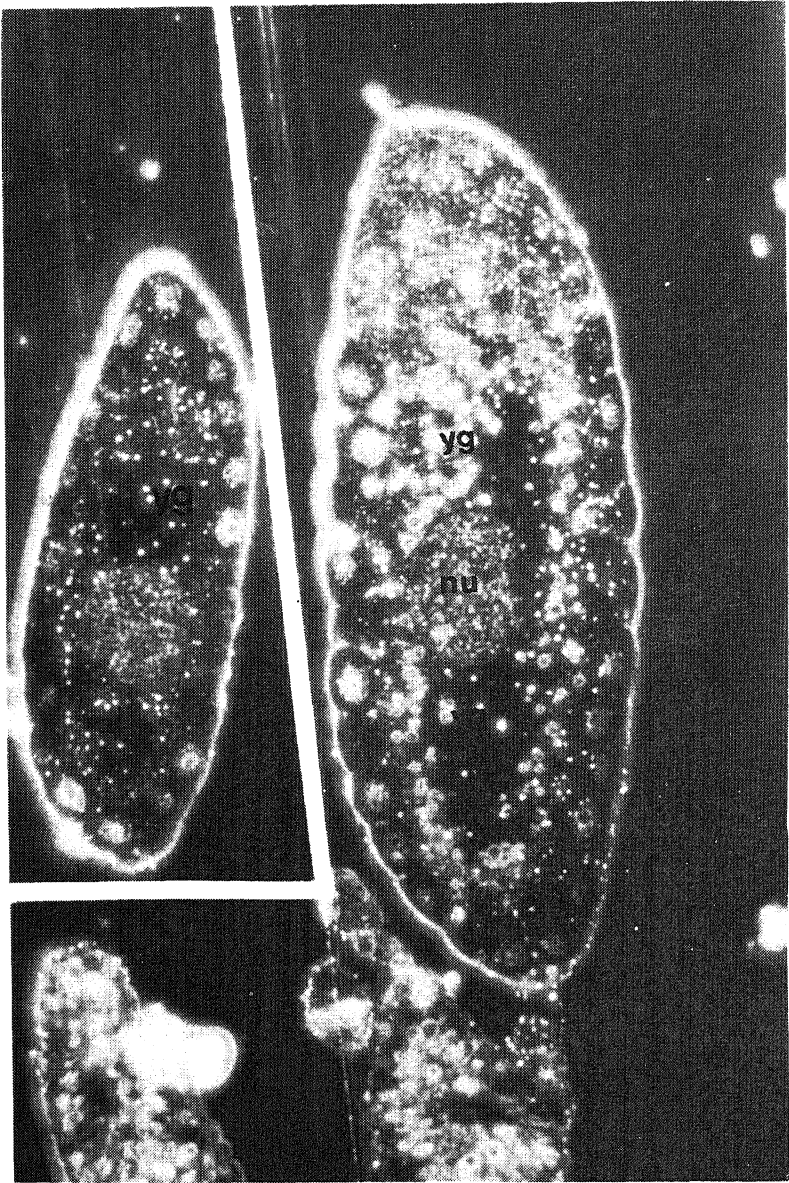
Figure 1. Dark field picture of the immature and mature oocytes showing small yolk granules dispersed among larger yolk spheres (× 1250).

polysomes (figure 6B). The cortical ooplasm of the terminal oocyte becomes narrowed with a tendency of the yolk spheres to become larger and empty vesicles are also seen both in the central and cortical ooplasm. As the oocyte cortex becomes active in pinocytosis and formation of cortical oospheres, the perivitelline space develops. The close apposition of yolk spheres in a mature terminal oocyte has contributed towards a reduction or disappearance of vesicles (figures 6A and 7B). From the onset of vitellogenesis till its completion, the follicle cells by virtue of the
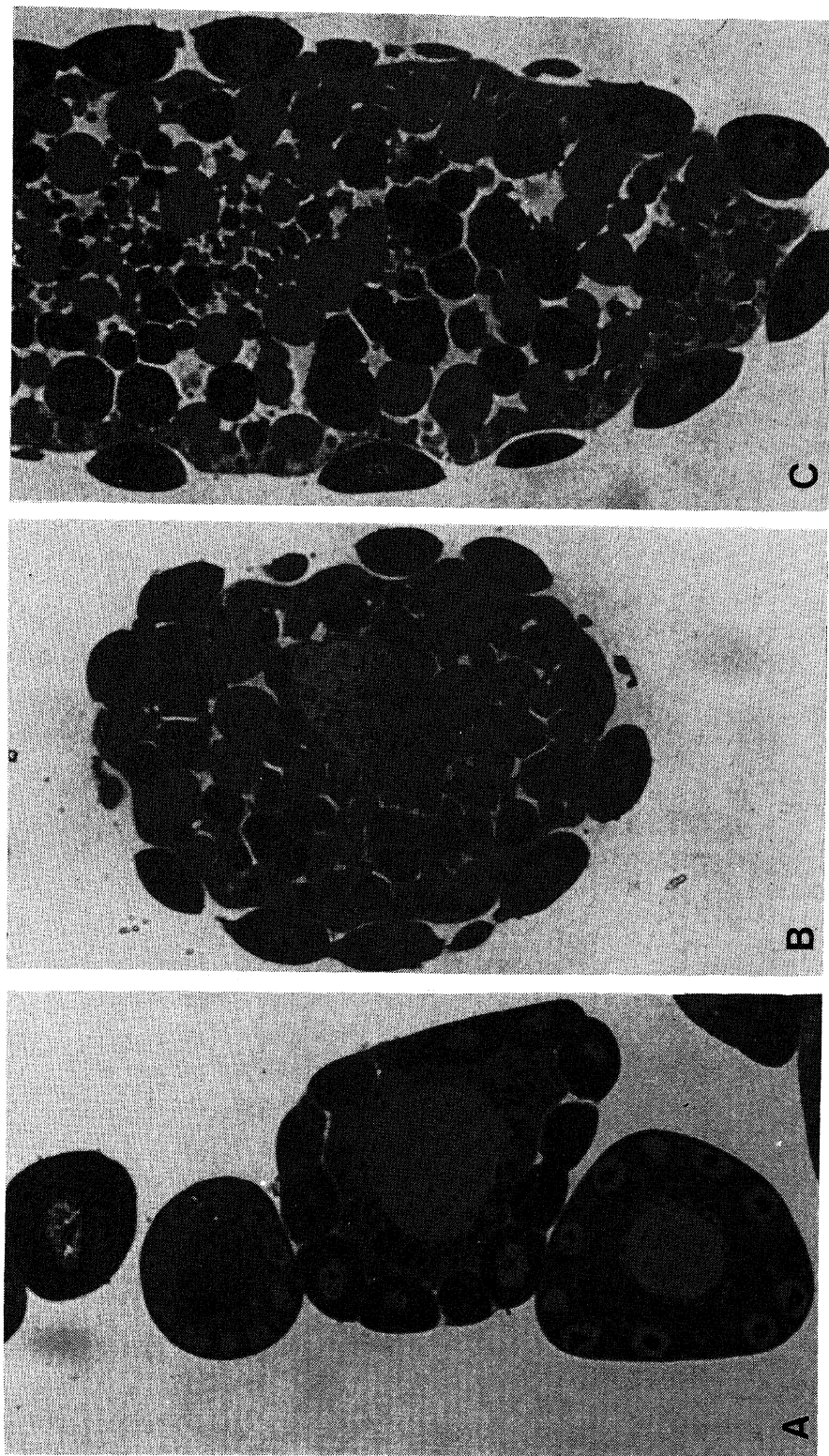
Figure 2. A. Cross section of developing oocyte showing the lipid droplets and refractile particles. **B.** Cross section of mature oocyte showing the protein and lipid yolk and smaller yolk granules. **C.** Longitudinal section of mature oocyte showing the protein and lipid yolk and smaller yolk granules. (× 1500).

Figure 3. The follicular cell-cortical ooplasm interface with a membrane showing several pore-like structures.

numerous organelles, they contain such as the mitochondria, golgi bodies as well as the rough endoplasmic reticulum and polysomes (figure 7A), progressively increase their capacity to synthesize and secrete proteins (figure 9). Further the mitochondria which were very numerous and conspicuous organelles found in the ooplasm during early previtellogenesis, further increase in number, growing in size as the oocytes grow and in late previtellogenic stages become dispersed into the cortical and subcortical ooplasm (figure 10). The follicle cells change their shape from the elongate to the spindle-shaped appearance as the oocyte matures and these changes result in large intercellular spaces between the follicle cells and the oocyte (figure 11). The follicle cells located at the anterior pole of the mature oocyte are somewhat
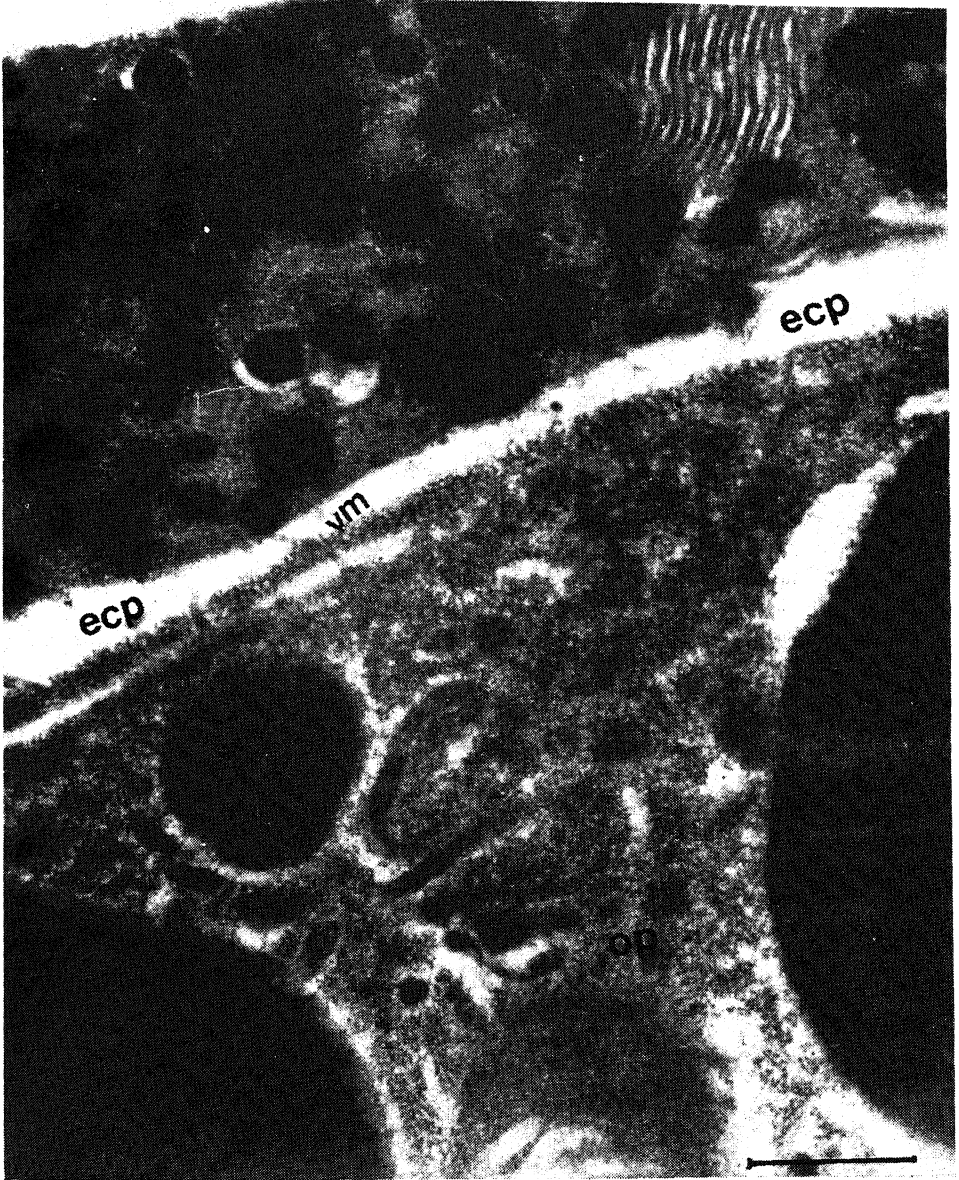
Figure 4. The widened follicular cell-oocyte interface as a result of rapid vitellogenic growth.

larger than the other cells and appear to send inter digitating membranous processes to the oocyte.

4. Discussion

Changes in the follicular epithelial cells appear to be well integrated with the differentiation of the oolemma and oocyte cortex becoming competent to incor-
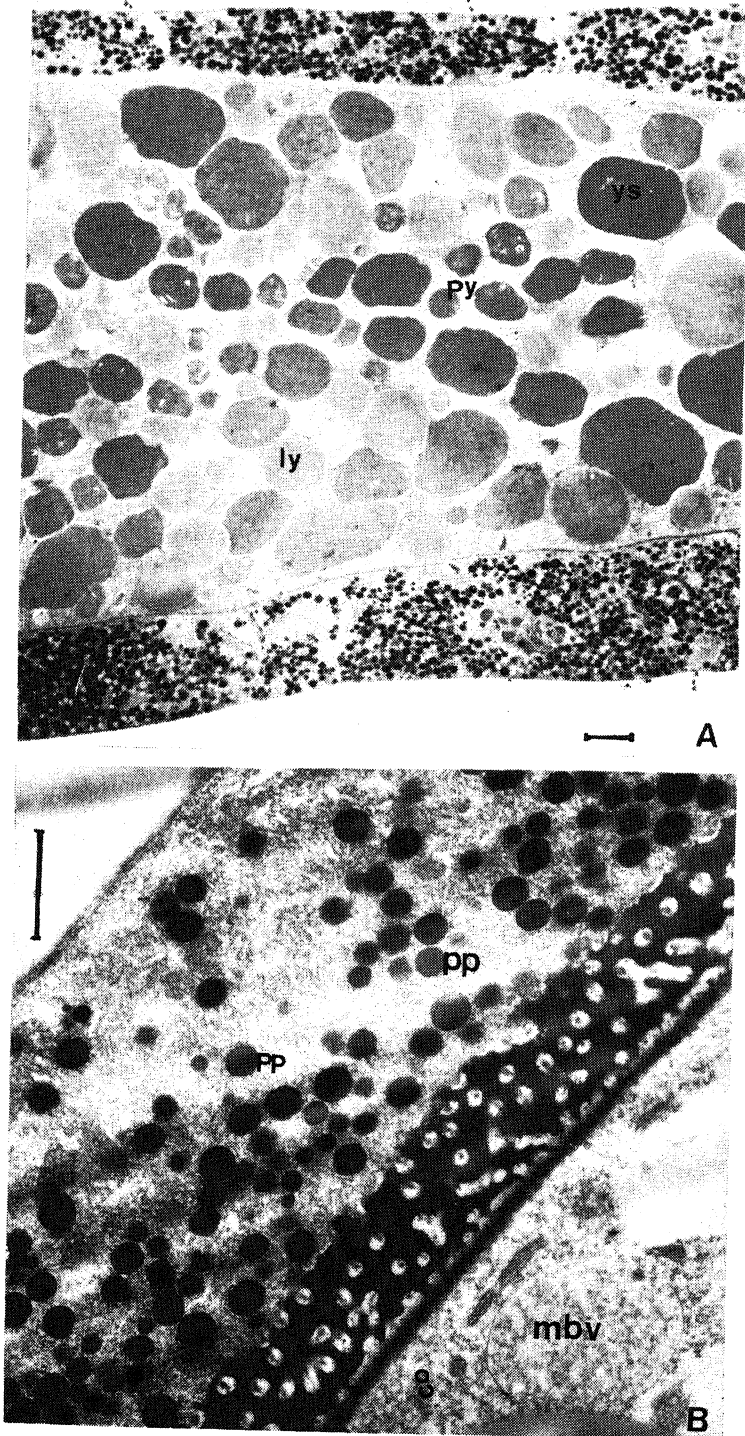
Figure 5. A. Ooplasm and follicular epithelium with different types of yolk spheres. **B.** Membrane bound vesicle in the cortical ooplasm.
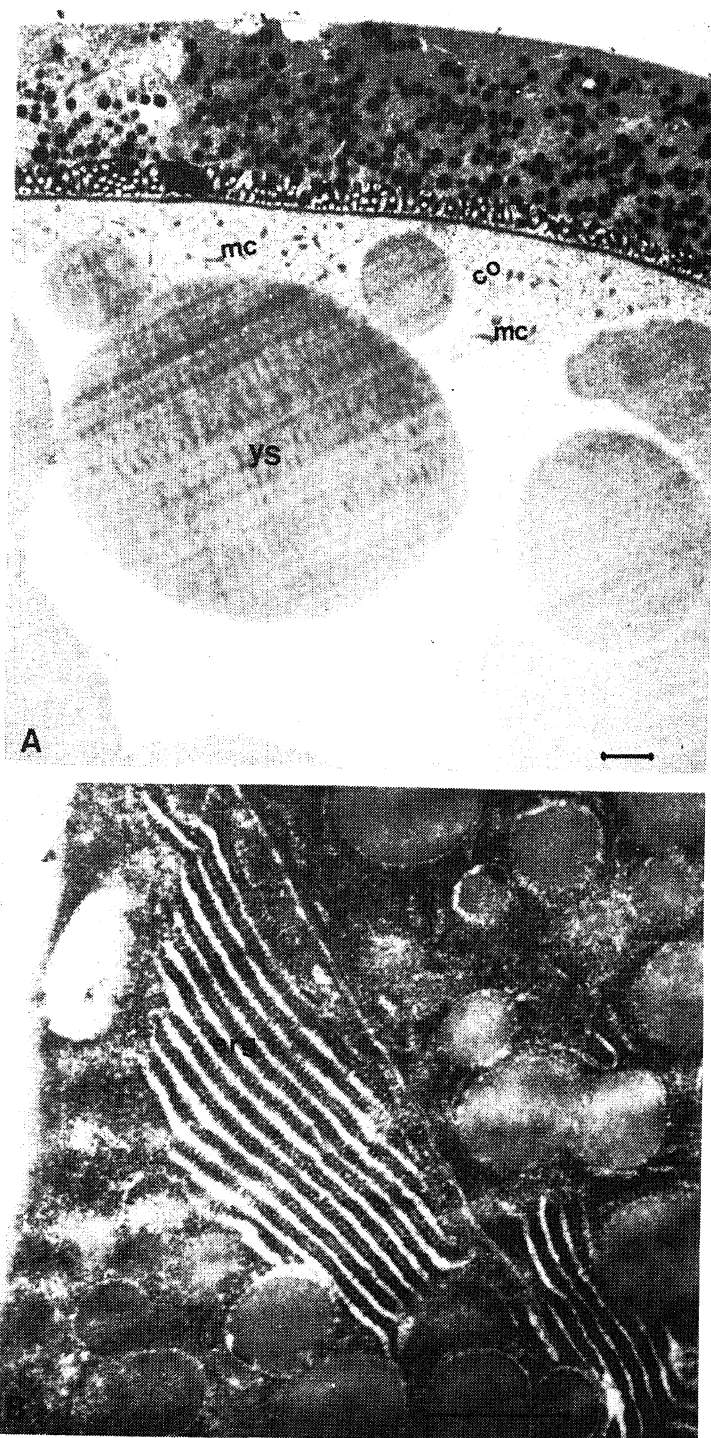
Figure 6. **A.** Decrease in cortical ooplasm as a result of increase in the number of yolk spheres, numerous mitochondria visible. **B.** Oocyte showing the endoplasmic reticulum with polysomes and numerous yolk-spheres.
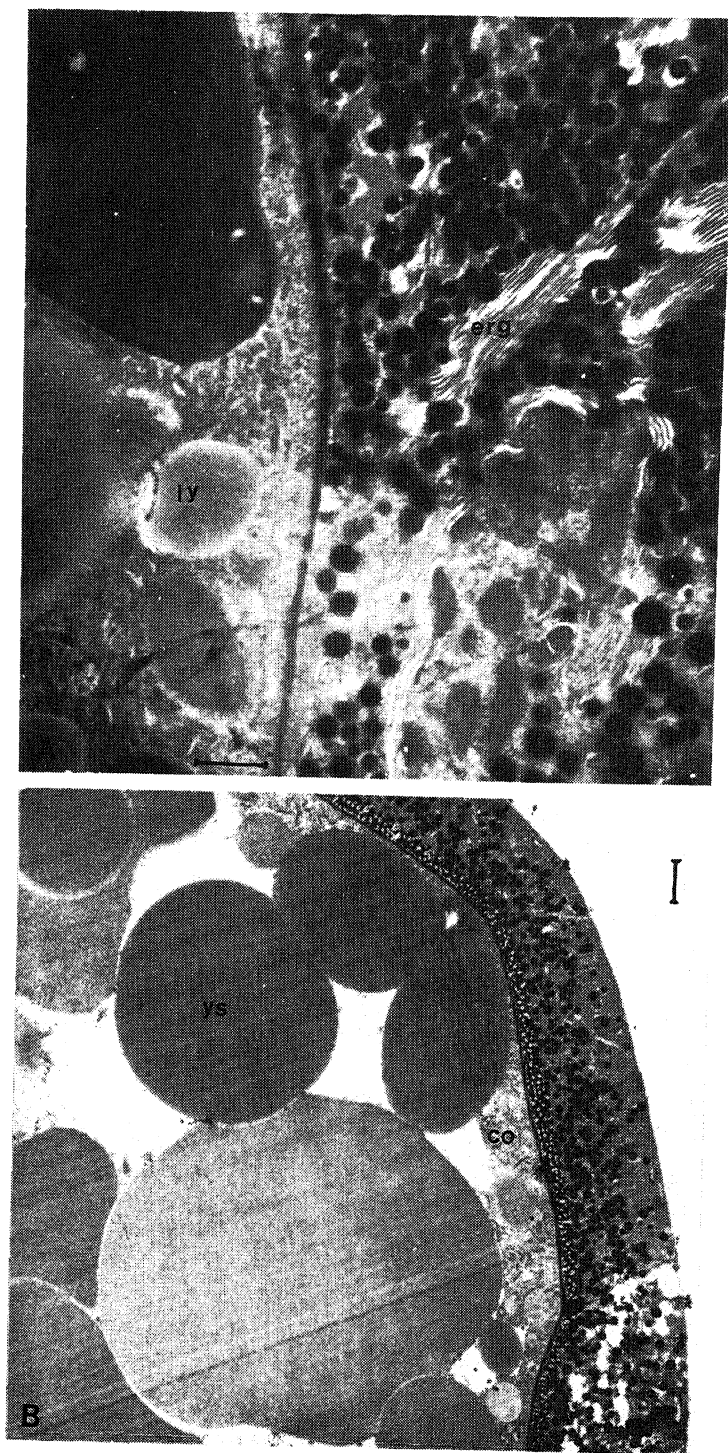
Figure 7. A. The follicular cell-oocyte interface showing the endoplasmic reticulum, protein and lipid yolk, and other organelles like mitochondria in the oocyte cortex. **B.** Aggregation of yolk spheres and incidental reduction in cortical ooplasm.
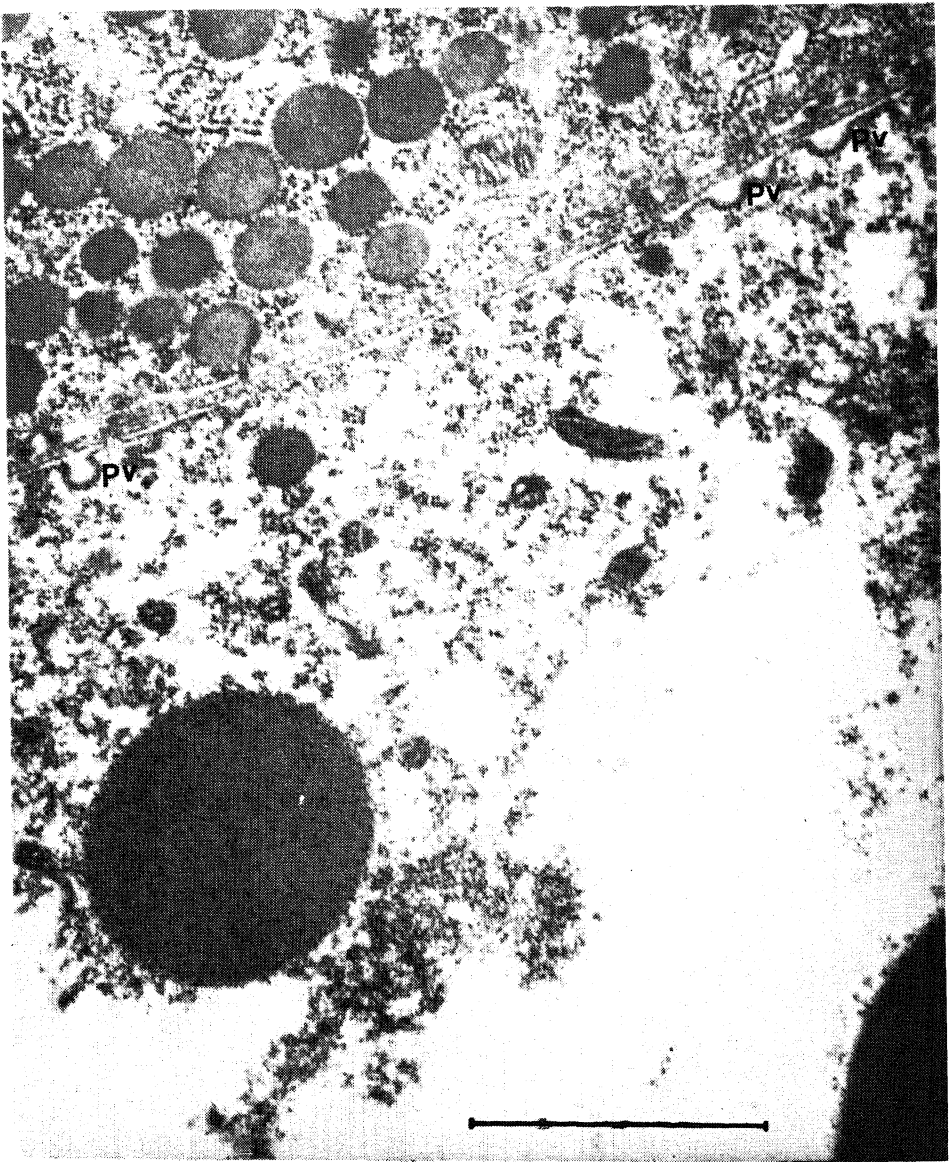
Figure 8. Pinocytic vesicles at the follicular cell and oocyte cortex interface.

porate yolk precursors (Huebner 1981). Localisation of the initial appearance of yolk spheres at the oocyte cortex is an aspect reported in many insects and subsequent to Bonhag's (1958) review, some of the major contributions in this direction relate to those of Bonhag and Arnold (1961), Telfer (1961, 1965), Telfer and Melius (1963), Telfer and Smith (1969), Giorgi and Mazzini (1984) and Mazzini *et al* (1986) and these have added considerably to our understanding of follicle cell-oocyte interactions. The mechanism involving the pinocytic origin of proteins through the formation of vacuoles has been stressed by Telfer (1965) as occurring by the pulling into the cyto-
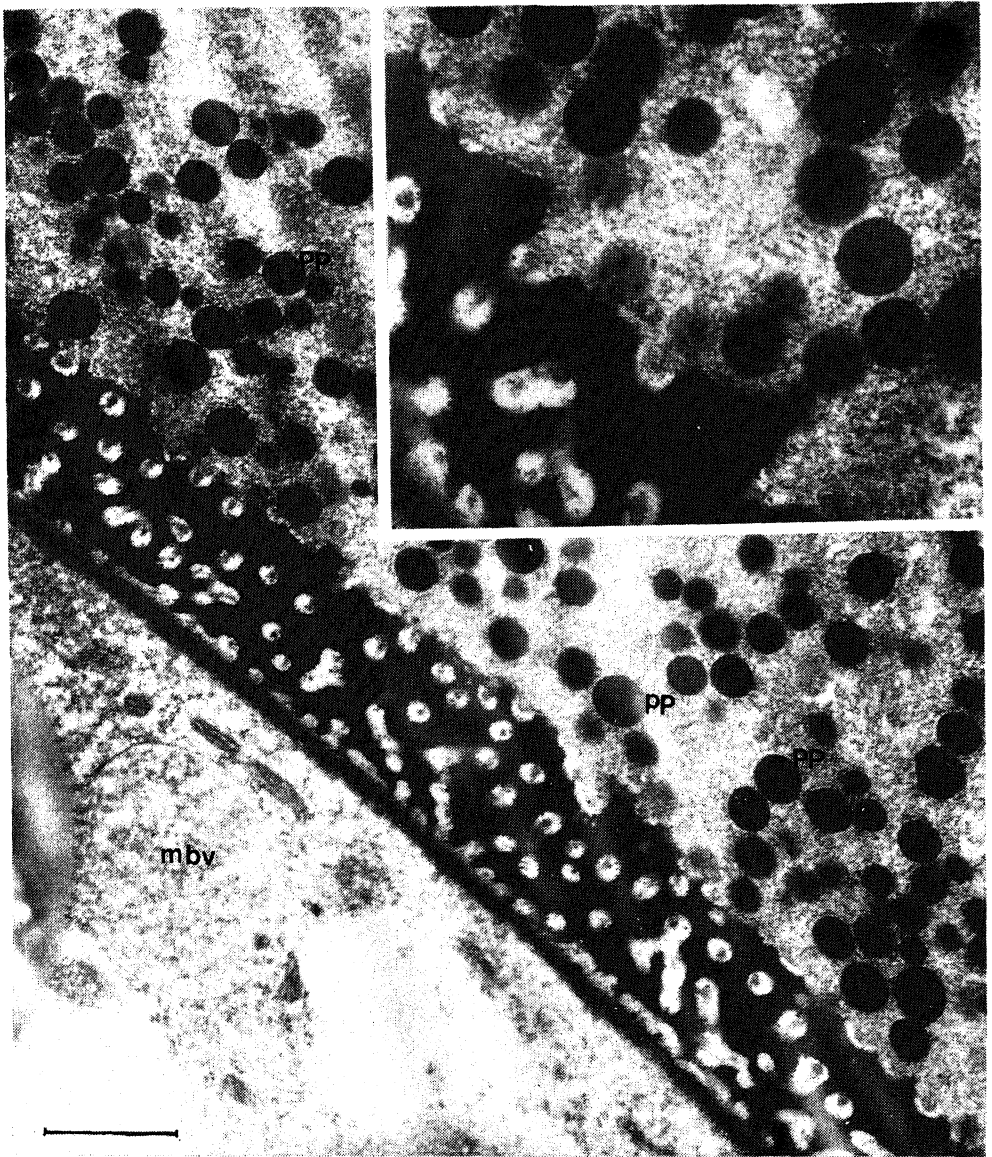
Figure 9. Showing the condensed protein precursors in the follicular cell with a portion enlarged to show the nature of the secretion.

plasm, a vesicle surrounded by a cell membrane enclosing a drop of extracellular fluid. TEM studies on the oocytes of *Arrhenothrips* have confirmed this, as also the fusion of vesicles and yolk spheres. It is also evident from the structure of the follicle cell—oocyte interface discussed earlier, that yolk precursors either passing through or originating in the follicle cells will have to pass through a significant intercellular gap (Telfer 1961).

The occurrence of electron dense vesicular bodies in the early vitellogenic follicles appear to be the result of degradation of aged cell organelles and Mazzini *et al* (1986)
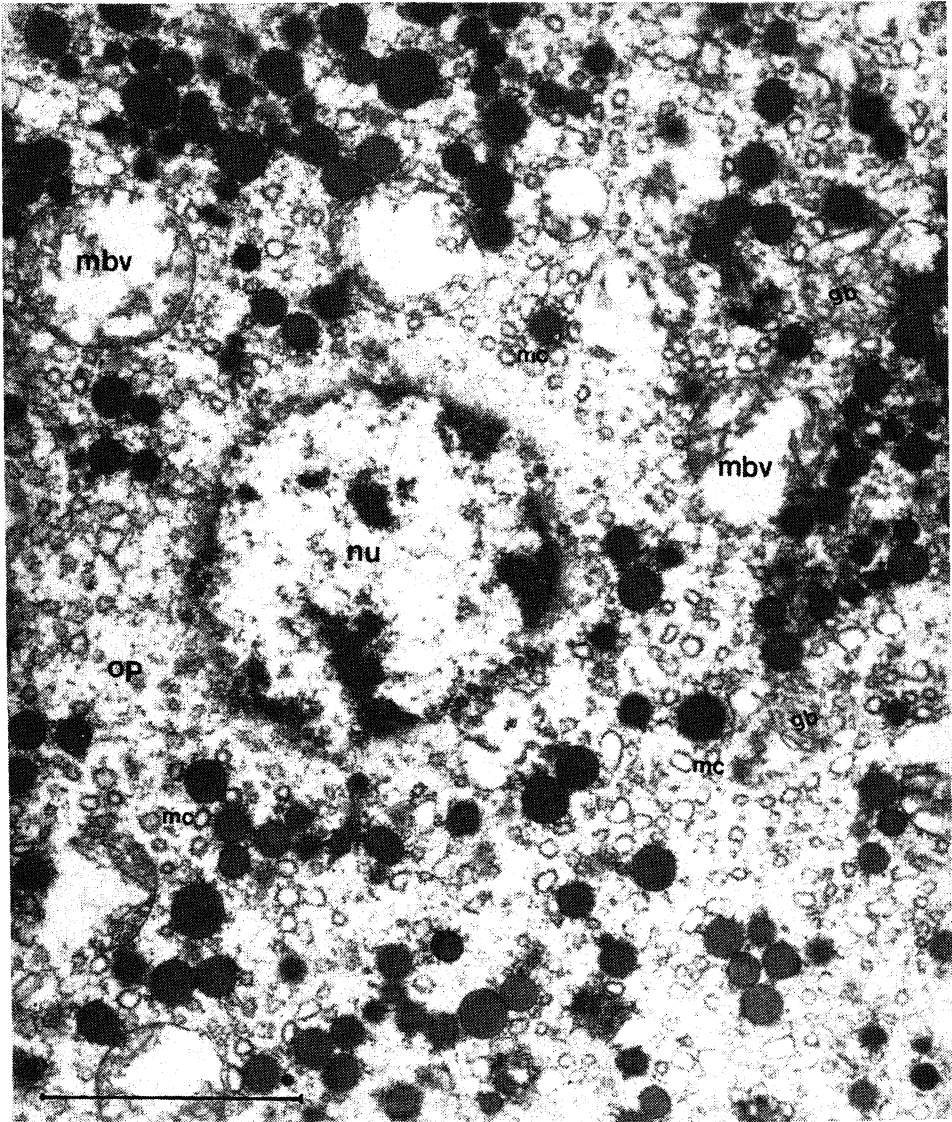
Figure 10. Phenomenal increase in the mitochondria and other organelles as well as membrane bound vesicles during vitellogenesis. Note the large nucleus and golgi bodies.

have interpreted that the mitochondria are presumed to condense into electron dense material. The fact that yolk-like vesicular bodies appear to fuse with endocytically derived vesicles to yield fully formed yolk spheres appear consistent with the present

Figure 11. **A.** A mature follicular cell showing condensed precursors. **B.** The change of shape into spindle like nature in post-vitellogenesis. (All bars represent 10 μm).

(Abbreviations: co, Cortical ooplasm; ecp, extra cellular space; erg, ergastoplasm; fe, follicular epithelium; gb, golgi bodies; ly, lipid yolk; mbv, membrane bound vesicle; mc, mitochondria; nu, nucleus; op, ooplasm; pr, pores; pv, pinocytic vesicle; py, protein yolk; vm, vitelline membrane; pp, protein precursors; ys, yolk sphere).
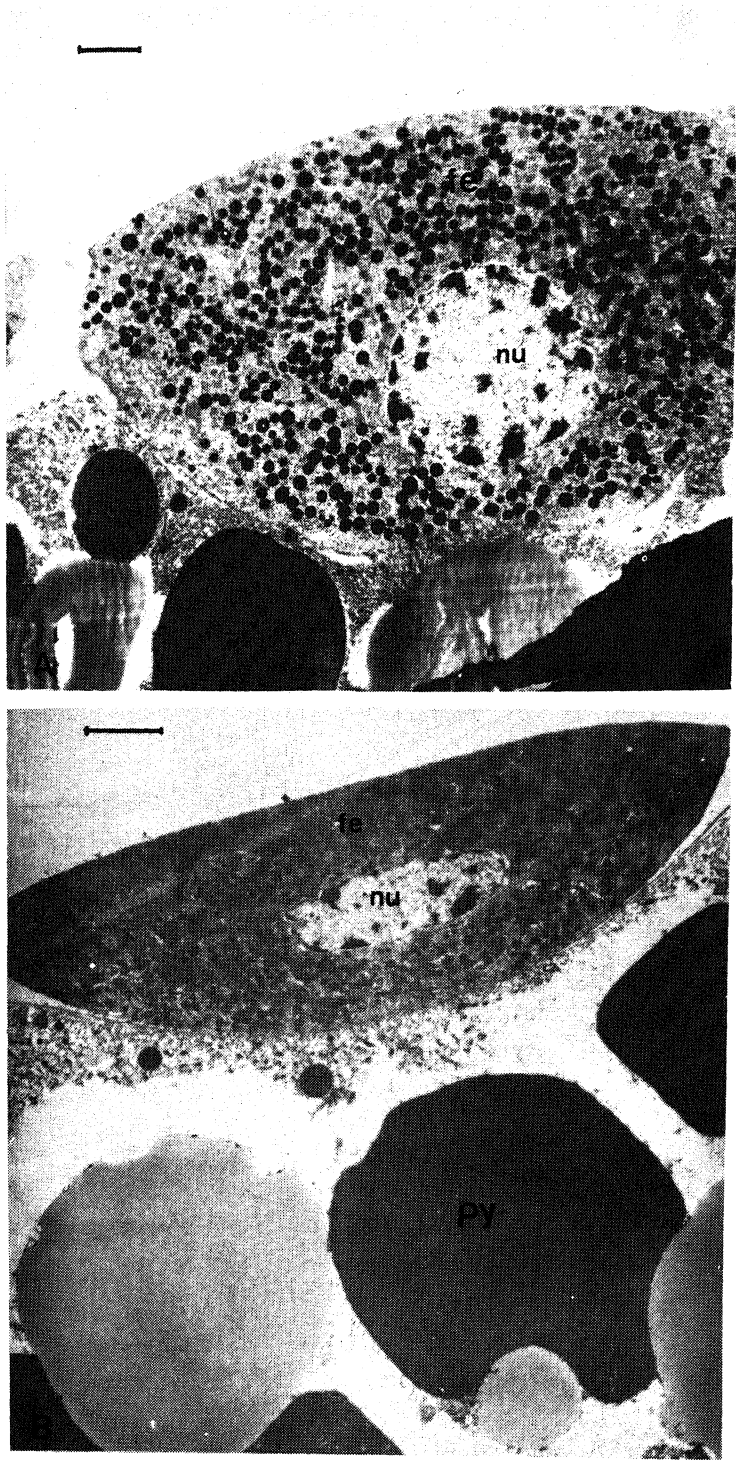
Figure 11.

observations. That these previtellogenic yolk-like vesicular bodies contribute to the yolk spheres of later stages has also been demonstrated for the panoistic ovary of *Bacillus rossius* (Giorgi and Mazzini 1984). Whether all the vesicles close to the yolk spheres are endocytic is not clear, since the possibility of some of them being golgi derived vesicles (Giorgi and Jacob 1977) and some others being enroute to the oolemma following recycling from the yolk spheres (Telfer 1965) are aspects deserving further scrutiny. That the yolk spheres continue to coalesce until the mature oocyte gets completely filled with yolk with no yolk-free ooplasm left, has also been amply demonstrated in the mature oocyte of *Arrhenothrips*. Observations by Haga (1985) whose investigations on the oogenesis and embryogenesis of *Bactrothrips brevitubus* briefly indicate that as yolk deposition proceeds the follicular epithelium becomes more and more thin and is more expanded, the mature oocyte at this stage having numerous yolk globules and scarce protoplasmic reticulum and periplasm. In view of the absence of an alternate route for the entry of proteins, both the ovarian wall and the follicle cell epithelium have been found to have characteristic intercellular pathways during the period of protein yolk formation enabling free penetration of blood proteins (Telfer 1965). The transmission of blood proteins to the oocyte surface is completed during the terminal growth phase of the mature oocyte and the continuation of pinocytic activity at the oocyte surface during this phase is suggestive of the incorporation of follicle cell secretion within the oocyte. Accumulation of haemolymphatic material and their intense uptake by the oocyte leading to the formation of typical yolk globules has been well demonstrated in *Locusta migratoria* (Goltzene 1979). Unmistakably the follicle cells have a dual function of monitoring the entry of precursors as well as contributing synthetic products of their own to the oocyte. Further sequentially viewing the functional changes involved in egg formation, it may be indicated that following initiation of yolk production is the development of the vitelline membrane and subsequent transformation of the oocyte cortex into the periplasm, the future site of establishment of the blastoderm and finally the deposition of the chorion by the follicle cells. The change of shape and enlargement of some of the follicle cells at the anterior end, during the final phases of growth of the mature oocyte, as well as the development of interdigitating processes are aspects very typical of *Arrhenothrips*. Matsusaki *et al* (1985) in their studies on the panoistic ovary of the dobson fly *Protohermes grandis* also indicate that the follicular epithelium investing the anterior pole of the oocyte is significantly thick and has synthetic function of the micropylar apparatus and the egg membranes. Ananthakrishnan and Balu (1986) have reported in *Dolicholepta gracilipes* the enlargement of a single apical cell with a large, prominent nucleus which becomes differentiated and activated from the others and progressively increasing in size and establishing a cytoplasmic link with the oocyte and with the completion of yolk deposition, disintegration of cell was observed. While this is peculiar to *D. gracilipes*, the possibility of extensive behavioural diversity in the nature of the anterior follicle epithelial cells in other Thysanoptera cannot be overlooked.

Acknowledgements

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Effect of feeding, ageing and diapausing on longevity and oviposition in the adult females of the army worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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Abstract. Adult females are fed at regular intervals after emerging from the puparium, then forced to diapause at temperatures ranging from 5–10°C. Longevity, oviposition and the diapausing period are recorded. Ageing is indirectly proportional to the diapausing ability and directly proportional to the maturation of the gonads and oviposition.

Keywords. Diapause; feeding; longevity; oviposition; *Spodoptera litura*; Noctuidae.

1. Introduction

The army worm, *Spodoptera litura*, a polyphagous insect is a serious pest of several crops (Garard *et al* 1985). Moussa *et al* (1960) reported that the army worm feeds on 112 cultivated plants belonging to 44 families all over the world. Food plants play a vital role in development, survival and reproductive potential of insects (Painter 1951). In this communication, we report the relationship between the feeding and the diapausing ability, feeding-ageing and the oviposition effect and feeding-diapausing and the longevity period of the adult. The significance of the survival of the dia-paused insect is also discussed.

2. Materials and methods

The culture of *S. litura* was reared from the soybean fields of Asian Vegetable Research and Development Center (AVRDC), Taiwan, ROC, in 1986. The colony was maintained in a growth chamber, fabricated by the Long Light Co., Taiwan. They were maintained at 28 ± 1°C, 60 ± 5% RH and 12 h photophase. The artificial diet for *S. litura* was obtained from Bioserve Inc., French Town, New Jersey, USA (Bio-Mix 9787). Among the adults which emerged from the puparium, only females were used for the experiment. Each female was placed in a plastic container and was fed with 10% honey solution. Feeding of the females was carried out according to the purpose of the experiment.

Experiment-I: A total of 20 females were divided into 4 groups of 5 insects each. The first group was fed daily with 10% honey solution for 3 days. After 3 days, they were made to diapause between 5 and 10°C temperature. After the diapausing period of 7 days (which was kept constant for all the experimental insects), they were brought to room temperature to find out if they are diapausing or not. Then they were maintained in the growth chamber and fed as before. Diapausing period, longevity, oviposition and mortality were recorded. The same procedure was followed for the second group of females. The procedure for the third and fourth

groups was adopted except the females which were fed on alternative days. The females of the third group were fed on the 1st, 3rd, 5th and 7th day and the females of the fourth group were fed on the 1st, 3rd, 5th, 7th and 9th day. For the oviposition, tissue paper was introduced into rearing containers.

Experiment-II: Ninety adult females of *S. litura* were divided into 6 groups, each containing 15 females. The zero day group was not fed and they were made to diapause on the same day of emergence. The females of 1, 2, 3, 4 and 5 days were made to diapause after they had completed their respective days in the growth chamber where they were fed once a day with 10% honey solution. The diapausing period was not predetermined and was 5–10°C temperature. When they were diapausing, they were brought to room temperature once a day to find out if they are alive or not.

3.   Results

Figure 1 shows results for the females which are fed daily for 3 days. From the results it is clear that two females lived beyond 4 days after diapause and one of them also laid eggs (which is shown in figure 5), while two others lived 2 and 4 days respectively after diapause. The remaining two females died during diapausing period.

   Figure 2 shows results for the females which are fed daily for 5 days. Among them, one female lived beyond two days after diapause, while another lived just for one day after diapause. Mortality of the remaining 3 females occurred during diapausing period.

   Figure 3 shows the results for the females which are fed on alternative days viz. on the 1st, 3rd, 5th and 7th day before they were forced to diapause. The mortality of all females occurred during the diapause period, but 3 of them laid eggs (figure 5) 1 h after they were forced to diapause and consequently died during diapause.

Alive days / No. of Insects	Normal conditions (in growth chamber)	Diapausing period							Normal conditions (in growth chamber)					
	01 02 03	04	05	06	07	08	09	10	11	12	13	14	15	16
2	▬▬▬	• • • • – – –												
1	▬▬▬	• • • • • • • • • • • • • • • • •						▬▬						
1	▬▬▬	• • • • • • • • • • • • • • • • •						▬▬▬▬▬						
1	▬▬▬	• • • • • • • • • • • • • • • • •						▬▬▬▬▬						

Figure 1.   Life longevity tables of daily fed females for 3 days, followed by a week of diapausing period (kept constant for all the females of experiment-I) and to returning normal conditions.

Alive days / No. of Insects	Normal conditions (in growth chamber)	Diapausing period						Normal conditions (in growth chamber)				
	01 02 03 04 05	06	07	08	09	10	11	12	13	14	15	16
3	▬▬▬▬											
1	▬▬▬▬	• • • • • • • • • • • • • •	▬									
1	▬▬▬▬	• • • • • • • • • • • • • • •	▬ ▬ ▬ ▬									

Figure 2.   Life longevity tables of daily fed females for 5 days, followed by a week of diapausing period and returning to normal condition.

(3)

Alive days / No. of Insects	Normal conditions (in growth chamber) 01 02 03 04 05 06 07	Diapausing period 08 09 10 11 12 13	Normal conditions (in growth chamber) 14 15 16
5	▬▬▬▬▬▬▬		

(4)

Alive days / No. of Insects	Normal conditions (in growth chamber) 01 02 03 04 05 06 07 08 09	Diapausing period 10 11 12 13 14 15	Normal conditions (in growth chamber) 16
1	▬▬▬▬▬▬▬▬		
4	▬▬▬▬▬▬▬▬		

Figures 3 and 4. Life longevity tables of alternative (1, 3, 5, 7) days fed females with a week of diapausing period and returning to normal conditions.

▲ Laid eggs
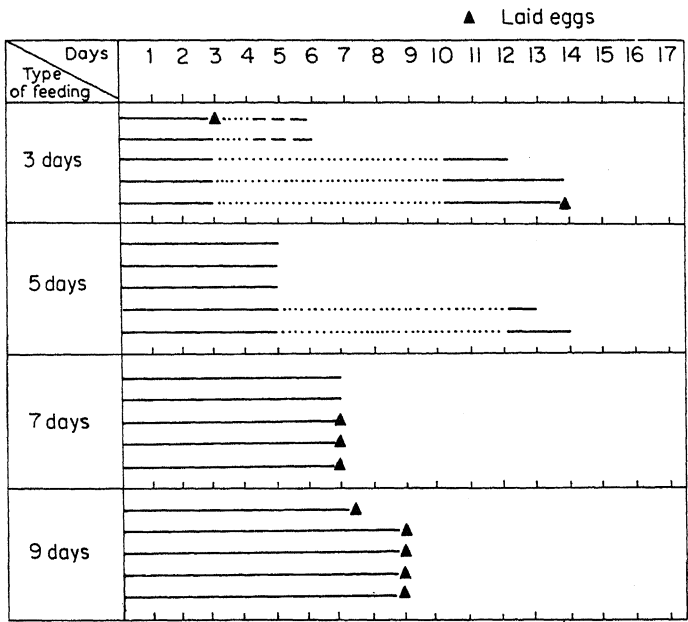
Figure 5. Life longevity tables of all the females involved in experiment-I (figures 1–4) with all the treatments showing oviposition, diapause, longevity and mortality.

(Note: Straight line indicates normal conditions before diapause; dotted line indicates diapause period; dotted-straight line indicates normal conditions after diapause; blank space indicates mortality; broken line indicates alive beyond).

Figure 4 reveals the results for the females which are fed on alternative days viz. on the 1st, 3rd, 5th, 7th and 9th day. Among them, one female laid eggs on the 8th day and died in the growth chamber itself even before diapause. The remaining 4 females were made to diapause. It resulted that all of them laid eggs 1 h after they were put in diapause and consequently died in diapause (figure 5).

Figure 5 overviews all the parameters used in the experiment-I whether it was in the growth chamber or in the diapausing equipment and reports the results. The egg laying behaviour of all the experimental insects are indicated in this figure.

Table 1 reveals the presence of the longest diapausing female in each group. It also indicates the average period of diapause of an insect in each group. It is important to note that the insects that were fed for 3 days have the highest average of diapause (4 days/insect) among 90 females involved in the whole experiment. On the opposite, the lowest average (1·32 days/insect) is held by insects from the zero day feeding group.

Figure 6 shows the longest diapausing female in each group and the average period of diapause of any individual female of that group.

Table 1. Life diapause tables of 90 females for all treatments, showing the longest diapausing period, average diapause period and the mortality of all the females involved in the experiment-II.

Treating days Sum of dead insects Alive days	0	1	2	3	4	5
1	11	3	5	5	2	1
2	3	2	4	0	0	3
3	0	3	1	3	7	7
4	0	0	0	2	1	1
5	0	4	2	0	4	3
6	1	2	2	2	1	
7		1	1	2		
8				0		
9				0		
10				0		
11				0		
12				1		
The longest life	6	7	7	12	6	5
The average period of diapause	1·53	3·66	3	4	3·53	3·13

Temperature range 5°–10°C is kept constant.
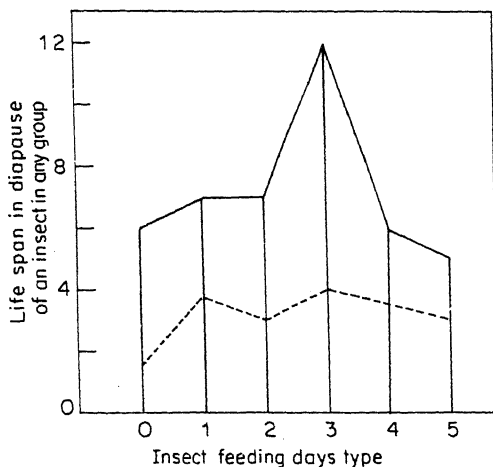
Figure 6. Straight line indicates the longest diapausing period of female of each group while the dotted line indicates the average period of any female of each group.

4. Discussion

Diapause is a time of slowing or stopping development, feeding and all but few maintenance functions. It involves arrest of development and this means in the adult, to prevent the maturation of the gonads (Horn 1976). Diapause may occur in the eggs, larvae, pupae or adult stages, though most commonly it occurs in the egg or pupa. There has been numerous reports of pupal diapause studies in the Lepidoptera species (Jayaraj 1981; Hachett and Gatehouse 1979; Dingle 1978; Roome 1979). Practically, there is very little information available on the adult diapause studies. It has been reported that diapause in the pupae of *Heliothis armigera* has been found to be induced by temperature and short day lengths (Roome 1979). All the females of *S. litura* that were made to diapause were all under low temperature condition i.e. 5–10°C. Feeding has been shown to promote mating and oviposition in *Heliothis* species (Parsons and Marshall 1939; Callahan 1958). Feeding with age leads to maturation of gonads and oviposition in the adult females of *S. litura* (figure 4). The eggs were laid during the feeding period when gonads obviously become mature (figures 3 and 4). Since the females had no chance to mate with the males, they oviposited only unfertilized eggs. It must also be said that all females that had laid eggs died subsequently 1 h after laying eggs. The results (figure 5) clearly indicate that the ability to diapause and be alive afterwards is much higher with the younger females than with the older ones (figures 1 and 2). But feeding with the age has decreased the capacity for diapause. Feeding has evidently been not a factor to increase the diapausing capacity (figures 2–4). In the final analysis it is the age factor of the females that weighed in favour of diapause rather than the feeding factor of the females. Greater the feeding is higher the metabolic activity in the adult, which is a hindrance to diapause of adults. It can be inferred that initial feeding (moderate) is more than sufficient for the diapause. The whole thing can be summed up in this way that aging and feeding is directly proportional to the maturation of the gonads and oviposition effect while the young female (age) with initial feeding is directly proportional to the diapausing capacity. Ageing certainly inhibits the diapausing process.

Mass rearing of insects have been successfully done to enable greater experimental studies in the field of applied entomology. Adult diapausing studies can then be very useful in such experimental studies, especially in delaying the use of insects for varying conditions particularly with respect to temperatures and day and night conditions.

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Faecal weight as an index of development rate and energy content of imago of lepidopterous insects

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Abstract. Statistically significant correlation coefficients were obtained for the relation between defecation rate and development rate as well as energy contents of pupa and imago of the noctuid moth *Achaea janata*. Using simple regression equations, the dependent variables development rate, pupa and imago were predicted against values of defecation rate obtained in a factorial design of experiment. The predicted and observed values did not significantly differ from each other indicating the possibility of predicting these parameters from easily estimatable faecal weight. The validity of the concept was tested using the data available in literature.

Keywords. Faecal weight; development rate; pupa; imago; *Achaea janata*.

1. Introduction

An innate tendency of all organisms is to acquire and accumulate sufficient energy to sustain and propagate. Although food is considered to be a less limiting factor for herbivores than for carnivores, owing to rapid and wide population oscillations, herbivores are likely to be exposed to abundance and scarcity of food under natural conditions (White 1978). This has become true for most lepidopterous insects for whom natural selection seems to have set a premium for faster rates of consumption and utilization of food especially during the final instar larval period to tide over the non-feeding pupal and adult stages (Waldbauer 1968; Muthukrishnan and Pandian 1983a). Maximization of energy accumulation can be achieved either by enhancing feeding rate with simultaneous shortening of growth period or by increasing the efficiency of conversion of food (Calow 1977). Available information points out that insects facing restricted availability of food adopt the strategy of prolonging their feeding larval period and emerging as miniature adults (Muthukrishnan and Delvi 1974; Mathavan and Muthukrishnan 1976; Muthukrishnan *et al* 1978; Muthukrishnan and Pandian 1984). Beddington *et al* (1976) and Hassell (1978) have substantiated the dependence of development rate (expressed as the reciprocal or larval duration) and fecundity of predatory insects on prey density. Statistically significant correlations have been reported for the relations between food consumed and development rate as well as pupal weight in the armyworm *Pseudaletia unipuncta* (Mukerji and Guppy 1973) and *Mamestra configurata* (Bailey 1976). The present communication aims to examine whether faecal weight can be used as a reliable index of development rate (D_r) and energy contents of pupa (Pe) and imago (Ie) of lepidopterous insects. Prediction of these parameters especially for lepidopterous pests will find useful application in contemplating pest management strategies. Use of faecal weight for the prediction of bioenergetics components of the predatory wasp *Sceliphron violaceum* and a few lepidopterous insects has been already sub-

stantiated by Marian *et al* (1982) and Muthukrishnan and Pandian (1983b), respectively.

2.   Materials and methods

In a factorial design of experiment, freshly moulted final instar larvae of *Achaea janata* (Lepidoptera: Noctuidae) (hatched and acclimated at 22, 27, 32 and 35°C) were reared separately on wide range of restricted rations as well as *ad libitum* level on fresh leaves of the castor *Ricinus communis* at their respective acclimation temperatures. The chosen ration levels were 50, 100, 200, 400 and 500 mg fresh leaf/ larva/day. As the larvae receiving 50 mg restricted ration at 32 and 35°C suffered high mortality, the lowest ration at these temperatures was raised to 100 mg/larva/day. The highest submaximal ration at 22 and 27°C was 400 mg/larva/day; it was 500 mg/ larva/day at 32 and 35°C. Faeces egested and left over leaf bits were collected separately and dried to weight constancy at 80°C. The duration required for the completion of the final instar at the tested temperatures and feeding schedules was noted. Samples of fresh Pe and Ie from each feeding schedule and temperature were weighed and dried. Energy content of these samples was estimated in a Parr 1421 semi-micro bomb calorimeter. Considering the initial and final live weight of the larvae, defecation rate was calculated as mg dry wt/g live wt of the larva/day; the following formula was used:

$$\text{Defecation rate } (F_r) = \frac{\text{Faeces (mg dry wt) egested during the final instar}}{\text{Live mid-body wt (g) of the larva} \times \text{duration (day)}}.$$

D_r was calculated as the reciprocal of the final instar duration. Mean F_r of the larvae reared at the tested conditions was related to D_r, energy contents of the Pe and Ie and separate regression equations were developed. Using these equations, the dependent variables were predicted for the observed rates of defecation. Goodness of fit of the predicted values with the observed values was tested using the chi-square test (Zar 1974).

3.   Results

The simple correlation coefficient ($r = 0.954$) obtained for the relation between F_r and D_r of the final instar *A. janata* larva at the tested conditions was statistically significant ($P < 0.005$; $N = 25$). The following regression equation explains the relation between the two variables:

$$D_r = 0.03772 + 0.001453 \ F_r. \tag{1}$$

Values of D_r predicted for the observed F_r using this equation are provided in table 1. Several predicted values were close to the observed values. The total chi-square value obtained for the differences between the observed and predicted D_r values (table 1) was statistically not significant (chi-square $= 0.0394$; $P > 0.5$; $D_f = 22$) showing that the predicted values come from the population of observed data.

Although the correlation coefficients obtained for the relation between F_r, Pe and Ie at all the tested temperatures and feeding schedules were statistically significant,

Table 1. Observed (D_r1) and predicted (D_r2) values of D_r of final instar *A. janata* as functions of defecation rate (mg/g larva/day). D_r2 values were predicted by using the equation (1).

Temperature/ration[a]	F_r	D_r1	D_r2
22°C			
50	25·95	0·0658	0·0754
100	41·20	0·0885	0·0976
200	69·40	0·1370	0·1385
300	88·70	0·1538	0·1666
400	105·70	0·2000	0·1913
Ad libitum	123·80	0·2000	0·2176
27°C			
50	38·30	0·0952	0·0934
100	61·40	0·1220	0·1269
200	98·90	0·1818	0·1814
300	117·10	0·2222	0·2079
400	132·80	0·2300	0·2306
Ad libitum	170·40	0·2300	0·2853
32°C			
100	64·40	0·1250	0·1313
200	92·70	0·1786	0·1724
300	116·60	0·2326	0·2071
400	129·30	0·2500	0·2255
500	147·80	0·2788	0·2525
Ad libitum	161·60	0·2788	0·2725
35°C			
100	68·30	0·1250	0·1370
200	97·10	0·1887	0·1788
300	117·50	0·2439	0·2084
400	142·00	0·2703	0·2440
500	153·50	0·2500	0·2607
Ad libitum	168·70	0·2500	0·2828

[a] mg fresh leaf/larva/day; total chi-square value for D_r1 and $D_r2 = 0.0394$; $P > 0.5$ at D_f 22.

the regression equations given below were found to be less precise for the prediction of either of the dependent variables.

$$Pe \text{ (J/individual)} = 0.0856 + 0.0226 \, F_r; \quad r = 0.858 \tag{2}$$
$$Ie \text{ (J/individual)} = -0.0986 + 0.0141 \, F_r; \quad r = 0.830. \tag{3}$$

Therefore, separate regression equations were developed for the relation between F_r and Pe as well as Ie for each temperature and provided in table 2. Figure 1 presents the regression lines for Pe and Ie drawn by using the equations in table 2. Most of the observed values fall on the regression lines showing the adequacy of these equations for prediction purposes. Total chi-square values for the differences between the observed and predicted values at each temperature were also statistically not significant.

4. Discussion

Values on D_r and F_r for the final instar larva of the monarch butterfly *Danaus chrysippus* exposed to different restricted rations (Mathavan and Muthukrishnan

Table 2. Regression equations for the relation between F_r and energy content (kJ/individual) of Pe and Ie of *A. janata*.

Temperature (°C)	Regression equation	N	r	Equation number
22	Pe = +0·153 + 0·0331 F_r	6	0·990	2
27	Pe = −0·457 + 0·0264 F_r	6	0·993	3
32	Pe = −0·767 + 0·0277 F_r	6	0·984	4
35	Pe = −1·289 + 0·0300 F_r	6	0·988	5
22	Ie = −0·136 + 0·0218 F_r	6	0·996	6
27	Ie = −0·441 + 0·0168 F_r	6	0·988	7
32	Ie = −0·722 + 0·0180 F_r	6	0·982	8
35	Ie = −1·031 + 0·0190 F_r	6	0·982	9
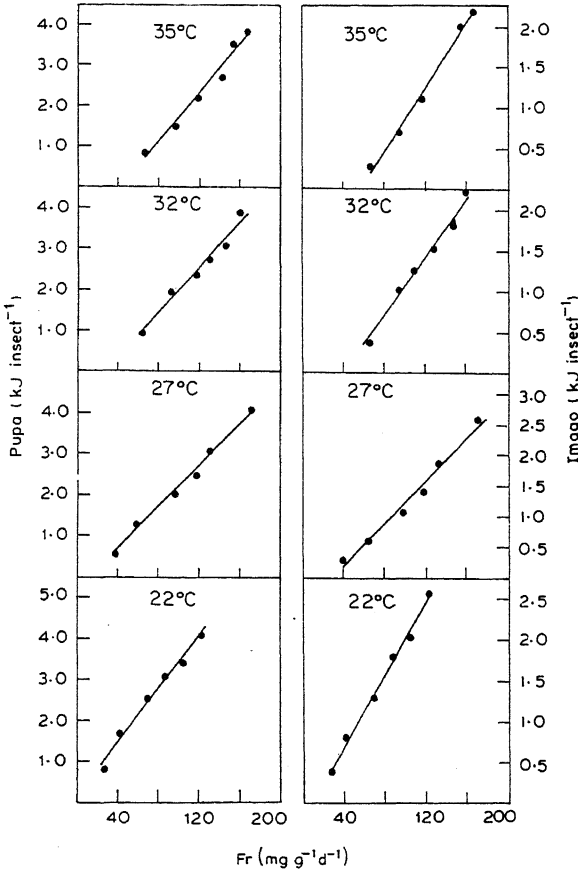
Figure 1. Energy content of Pe and Ie in relation to defecation rate (F_r in mg/g live larva/day) of *A. janata*. The lines were drawn using the regression equations (2–9) provided in table 2. Open circles indicate the observed values. Total chi-square for the difference between the observed and predicted values are:

22°C : Pe = 0·1611;   Ie = 0·02469
27°C : Pe = 0·1203;   Ie = 0·07490
32°C : Pe = 0·1577;   Ie = 0·07556
35°C : Pe = 0·1512;   Ie = 0·09720.

For all chi-square values $P > 0.5$ at D_f 4.

Table 3. Observed (D_r1) and predicted (D_r2) values of D_r of final instar larva of *D. chrysippus* (Mathavan and Muthukrishnan 1976) and *B. mori* (Muthukrishnan *et al* 1978) as functions of F_r (mg/g larva/day). D_r2 values were predicted from the equation given below (10 and 11).

Feeding schedule	F_r	D_r1	D_r2
Ration[a]		*D. chrysippus*	
200	84·0	0·1923	0·2023
300	107·4	0·2273	0·2304
400	116·8	0·2500	0·2471
500	177·4	0·3125	0·3143
750	177·9	0·3333	0·3149
Ad libitum	234·0	0·3704	0·3821
Feeding duration[b]		*B. mori*	
4	102·4	0·0862	0·0772
6	132·5	0·0909	0·1050
10	164·2	0·1333	0·1342
12	170·0	0·1429	0·1395
18	197·3	0·1667	0·1647
24	198·8	0·1667	0·1660

Total chi-square value for D_r1 and $D_r2 = 0.00223$; $P > 0.5$ at D_r 4
$D_r2 = 0.10171 + 0.0012 F_r$; $r = 0.986$ (equation No. 10).
[a] mg fresh leaf/larva/day.
Total chi-square value for D_r1 and $D_r2 = 0.0032$; $P > 0.5$ at D_f 4
$D_r2 = 0.01714 + 0.000921 F_r$; $r = 0.976$ (equation No. 11).
[b] h/day.

1976) and the silkworm *Bombyx mori* restricted to feed for different durations (Muthukrishnan *et al* 1978) were used to test the validity of the concept of predicting D_r from F_r. Table 3 provides the observed and predicted values of D_r against F_r of *D. chrysippus* and *B. mori*. The close proximity of the predicted values with the observed values and the lack of significance of the chi-square values for the differences between them reaffirm the dependance of D_r on F_r. The relation between the two variables implies that processing of some amount of food in the gut is essential for development to proceed. However, the positive intercept on $Y(a = 0.03772)$ of the regression equation (1) indicates that development is possible to a certain extent even in the complete absence of food consumption and faecal output. Relating the D_r of the spider *Linyphia triangularis* to food consumption rate, Turnbull (1962) also obtained a positive intercept on Y indicating that development can be proceeded even without food consumption. But, Beddington *et al* (1976) are of the view that under these conditions the slope and intercept of the line have no simple biological interpretation (Mukerji and Leroux 1969). Although Mukerji and Guppy (1973) and Bailey (1976) have reported significant correlations between food consumption rate during the larval period and the initial or final dry weight of the Pe for a few lepidopterous insects, it is not possible to make use of their data to find support for the prediction of Pe and Ie from F_r, as details of faecal weight and energy content of the Pe and Ie have not been provided by these authors.

Acknowledgement

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Predatory behaviour of *Rhinocoris marginatus* Fabricius (Harpactorinae—Reduviidae—Heteroptera—Insecta)

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Abstract. The pin and jab type of predatory behaviour of *Rhinocoris marginatus* Fabricius is distinctly different from the feeding behaviour of other subfamilies known so far. The thin long legs, tibial pads, long slender rostrum capable of more than 90° forward extension, slow gait of this predator are ideally suited for efficient capture of soft-bodied prey types, particularly caterpillars. As in other predatory reduviids, visual stimuli provided by the moving prey, elicit feeding responses in *Rhinocoris marginatus* and this is augumented by other sensory systems, including olfactory. The predatory feeding behaviour of *Rhinocoris marginatus* consists of disinct stimuli—response mediated sequences of events and based on these the feeding behaviour model for this insect is depicted.

Keywords. Predatory behaviour; Harpactorinae; Reduviidae.

1. Introduction

Studies pertaining to the prey records, feeding habits and feeding behaviour of predatory reduviids are meagre. Detailed predatory behaviour of a few representative species of Reduviidae from southern India (Haridass and Ananthakrishnan 1980a) indicated that these insects, inhabiting diverse habitats, feed on a variety of arthropod prey types. Since the feeding behaviour of *Rhinocoris marginatus* Fabr. a common harpactorine bug, varied considerably from those known already, an attempt has been made to study this aspect in detail, with the construction of appropriate feeding behavioural model depicting the sequences of events involved in predation.

2. Materials and methods

Adults and nymphs of *R. marginatus* were collected from Vaigai Dam (Madurai District) and Chandrapuram (Coimbatore District) of Tamil Nadu and from Konai falls (Chittoor District, Andra Pradesh) and maintained in cages under laboratory conditions (temperature 29°–32°C; RH 70–90%) similar to those explained earlier (Haridass and Ananthakrishnan 1980a). They were provided with grubs, maggots, grasshoppers and caterpillars; the caterpillar was found to be their natural food. During experiments the starved and highly cannibalistic forms were reared separately in appropriate plastic containers. Feeding behaviour of the insects were observed after the test insects were starved for 2–3 days and then offered with various prey types. Scanning electron microscopic (SEM) picture of the fore tibia of adults was obtained from fresh material using Hitachi 450A electron microscope after the materials were gold coated using Eiko IB.2 ion coater.

3. Results

As is true for other reduviids, the feeding behaviour of *R. marginatus* consists of many stimuli-response mediated sequence of distinct events and these can be broadly categorised into prey location, prey capture, prey immobilisation and prey consumption.

3.1 *Prey capture*

R. marginatus is always elicited by visual stimuli and a moving prey makes the starved and wandering predator more active. On locating the prey the predators raise the body from the ground and with the antennae directed forward, approach in slow and steady gait (figure 1A). When the prey is within the striking distance, well before the antennae could make any contact with the prey body, the rostrum is fully extended forward to about 90° and the fore legs are lifted off the ground, as if to pin down the prey (figure 1B). A dead caterpillar or an inert object dragged in front of the starved predator also evoke similar responses. Nymphal stages of these insects also behave in a similar manner.

3.2 *Prey capture and prey immobilisation*

Once the predator reaches the close proximity of the prey it attempts to pin down the prey with the tibial pads of the fore legs (figure 1C) and jabs at the prey body with its long rostrum and quickly injects the toxic saliva. Invariably the thorax or segments behind it are selected for salivary injection. When the caterpillar-prey is hairy, the tibial pads are not efficiently used for pinning down the prey and two to three probes with rostrum are required for site selection before insertion of stylets and injection of saliva. When bigger caterpillars are encountered, the predator merely jabs with fully extended rostrum and injects toxic saliva, without making any attempt to pin down the prey firmly with fore legs. The caterpillars attacked in this manner react violently and try to escape, but are pursued by the predator which repeatedly try to jab. Invariably bigger caterpillars manage to escape such assaults. Small and medium sized caterpillars also react violently when attacked by the predator by twisting and rolling their body and by releasing body and salivary fluids (figure 1D). In such cases the predator releases its hold, but with their stylets still inserted in the prey body, closely follow the wriggling prey that finally becomes immobilised and killed within 30–50 s. When the prey body size is small or as in the laboratory, when maggots and grubs were offered as food, the predator, after pinning down and injecting the saliva, completely lifts the prey off the ground (figure 1G). Unable to escape, the prey struggles dangling at the rostral tip until death. Nymphs of *R. marginatus* frequently attack the prey in groups; smaller 1st nymphs prefer newly hatched and very small caterpillars; 5–6 such nymphs are also able to kill a medium sized caterpillar easily. Three to five nymphal stages attack and immobilise medium and large sized caterpillars like the adults, but in groups of 2 and 3 (figure 1H).

When grasshoppers were offered as prey, the predator initially exhibited alarm responses by raising the body above the ground, by lifting the fore legs and by extending the rostrum and antennae forward. With continued visual stimuli, the
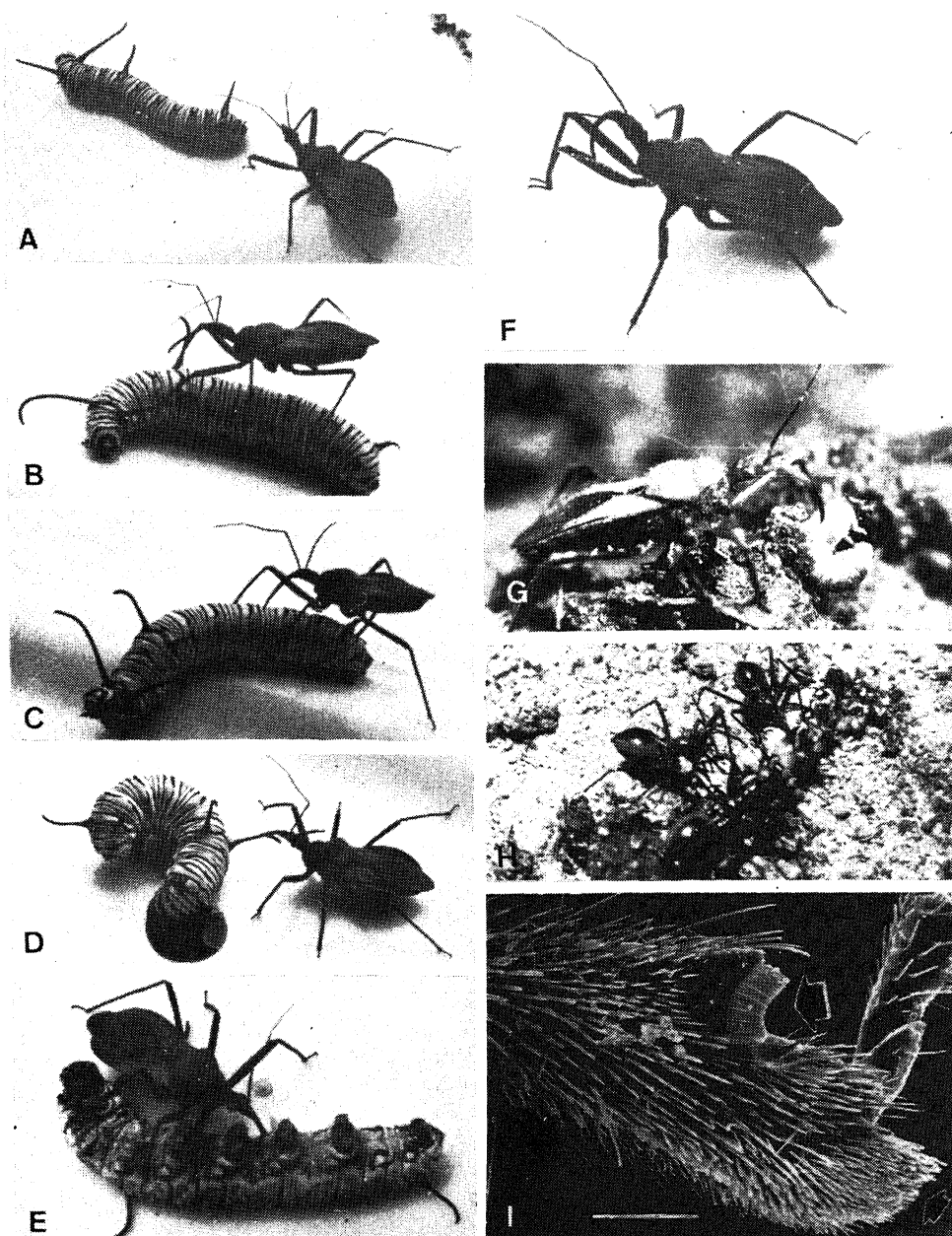
Figure 1. *R. marginatus.* **A.** Aroused by a moving prey the predator approaches with raised body, extended antennae, etc. **B.** The predator, after pinning down the prey, about to jab with rostrum. **C.** After selecting the suitable site, the predator jabs the prey body with rostrum and injects saliva. · **D.** Dragging the paralysed prey to a secluded place. **E.** Feeding the killed prey. **F.** Antennal cleaning with tibial combs. **G.** Predator feeding cow-dung beetle grub. **H.** Group feeding by nymphs on caterpillar. **I.** SEM picture of inner lateral view of fore tibial tip showing tibial comb (big arrow) and tibial pad (small arrow) (scale = 167 μ).

predator slowly approaches and using the fore legs, pins down the prey and tries
insert the stylets and inject saliva between the intersegmental membranes of t
abdomen or thorax. But in 7/10 cases the grasshoppers reacted violently on bein
touched or probed by the predator; they either walked away or jumped off to sa
places. On several occasions the grasshoppers were seen to kick the predator wi
the hind legs. Even the occasional successful prey capture of grasshoppers could
achieved by *R. marginatus* only when the prey were small nymphs or small size
adults (*Oxya nitidula* (Walker), *Cyrtacanthacris ranacea* Stol., *Euprepocnemis alac*
alacris Serv.–Acrididae). Bigger grasshoppers with powerful hind legs could not
captured by *R. marginatus*.

3.3 *Prey transport*

Transportation of the immobilised and killed prey to a secluded place before t
commencement of feeding is common in *R. marginatus*. When predation occurs
the ground, with the stylets still inserted, the predator walks backwards, dragging t
dead prey with the rostrum (figure 1D). Secluded places, under stones and crevices
the ground are usually selected. When predation occurs on plants, the dead prey
held at the rostral tip and easily carried to short distances before feeding actua
commences. When the prey is small, it is also held dangling at the rostral tip an
feeding may commence immediately after killing the prey. Dragging the killed prey
not seen in the case of nymphs that attack the prey in group.

Before dragging the prey for feeding, the predators spend considerable time to clea
their antennae off the body fluids and regurgitated salivary secretions (figure 1
released by the prey during attack. The antennal comb (figure 1I) on the inner upp
margins of the fore tibia are used for this grooming. After cleaning, the predat
reinserts the stylets into the prey body for transportation and subsequent feeding
the dead prey.

3.4 *Prey consumption*

This is the terminal part of the feeding behaviour and this may commen
immediately after killing or after transporting the prey to a secluded place, dependin
on the size of the prey as well as on the habitat of predation (figure 1E). Feeding sit
are never changed if the prey is small or if the prey is carried at the rostral tip, an
not frequently changed in the cases of larger prey. In all these cases feeding activity
terminated only after the full distension of the predator's abdomen. Fully satiate
adults show no feeding activity for the next 20–24 h.

Cannibalism is common in *R. marginatus*, smaller nymphs and adults, particular
those in the process of moulting, fall easy prey to large individuals. The variou
events and sequences of the feeding behaviour of *R. marginatus* are depicted in figu
2.

4. Discussion

The feeding behaviour of the exclusively predatory reduviids have been shown to
definitely correlated with the various prey types involved. Based on the sequences
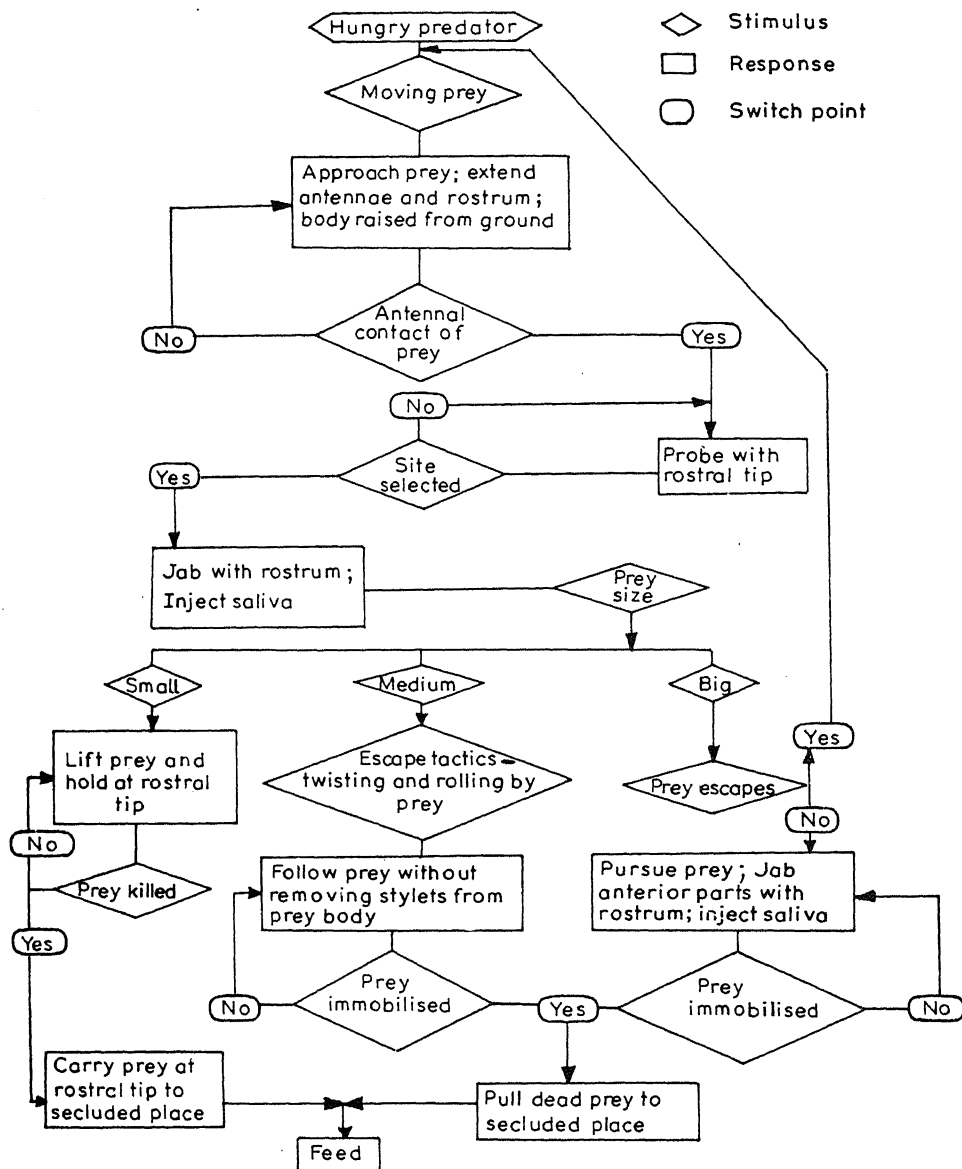
Figure 2. Flow chart depicting the predatory behaviour of *R. marginatus*.

events of their feeding behaviour, distinct behavioural models for the different subfamilies have been constructed (Haridass and Ananthakrishnan 1980a).

The feeding behaviour of *R. marginatus*, as is also true for most of Harpactorinae, appears to be different from other subfamilies studied and can be classified pin and jab type (Haridass *et al* 1987). The predators are characterised by the slow gait and by the possession of long, slender proboscis capable of more than 90° forward extension enabling them to attack soft-bodied prey types from a distance. Their legs are also very long, helping them to reach the prey quickly with a few strides. In

addition, their fore legs are also endowed with tibial pads to pin down the slow moving prey during immobilisation and killing.

Many insects, particularly honey bees, beetles, and bugs have been recorded a preys of Harpactorinae (Miller 1953, 1971; Parker 1972). But caterpillars of sever Lepidoptera have been reported to be the natural food for several Harpactorir species (Ambrose and Livingstone 1986; Beeson 1941; Bose 1951; Edwards 196 Hiremath and Thontadarya 1983; John Vennison and Ambrose 1986, 1987; Mill 1953, 1971; Nagerkatti 1981; Rao 1974; Swadner and Yonke 1973a, b). Ambrose an Livingstone (1985) have reported *R. marginatus* as a polyphagous predator of sever insects pests, particularly *Crytacanthacris succinata* (Acrididae). In the present stud however, caterpillars appeared to be the natural prey *R. marginatus*, and in th laboratory they exhibited preference to this prey than other prey types offere including maggots, grubs, ants, grasshoppers and termites.

Like in other predatory Reduviids, the moving prey offers important stimulus i the feeding behaviour of *R. marginatus*, arousing the predator from a state of akines to a high level of excitation. On perceiving continued prey movement, the predat orients towards the prey by raising the body and by extending the antennae in from slowly stalks the prey. The rostrum may be frequently fully extended during pursu When the prey is within the striking distance, even without the antennal contact, th forelegs are raised and used to pin down the prey body for subsequent stabbing wit the stylets and injection of toxic saliva. The sequence of events exhibited b *R. marginatus* in killing soft-bodied prey may be said to be: arousal—orientation— approach—antennal extension and contact—raising of fore legs—extension rostrum—pinning down prey with forelegs—insertion of stylets—injection of saliv Unlike the feeding behaviour of Piratinae, Ectrichodiinae and Triatominae (Harida and Ananthakrishnan 1980a), these behavioural sequences are not strictly follow in the case of *R. marginatus*. Their pin and jab type of behaviour, probably also most other Harpactorinae, varies in a number of ways. When the prey is small, it pinned down with fore legs and killed with the injection of saliva and complete lifted above the ground and held at the rostral tip. When the prey is large lil caterpillars, it is stalked for some time and subsequently pinned down and jabb with the rostrum. Though the struggling prey is released from its hold, the predat never removes the stylets from the prey body until the latter is fully immobilised ai killed. If relatively large and active like grasshopper, the predators stalk the prey ai then move away or repeatedly try to jab with rostrum at the escaping prey witho pinning down with fore legs; the chances of their successful prey capture in the cases being very little.

The visual stimuli provided by the moving prey play the important role inducing the feeding responses in *R. marginatus*. Since the behavioural sequenc varied according to the prey size and since the predator particularly selected t anterior body segments for stylet insertion and salivary injection, it is to concluded that the perception of the body form and the directional clue offered b moving prey are of primary importance to the reduviids. Antennal contact of t prey body appears to be not essential in most cases of successful predation and t reduviids do not normally touch any part of the prey before pinning and jabbir Hence the chemical stimuli involved should only be olfactory, and this wou augment only the later stages of the predatory behaviour. Impact of antennecton and eye-blocking experiments on the feeding behaviour of *R. marginatus* (Ambrose *al* 1983) are in conformity with the present observations.

Unlike Piratine and Ectrichodiine species, where active chasing and pouncing of the moving prey is involved (Haridass and Ananthakrishnan 1980a; Haridass *et al* 1987), most of the Harpactorinae including *R. marginatus*, slowly stalk the prey to pin and jab at it. Many morphological structures are evident for this kind of technique used in prey capture. These insects are endowed with long, slender legs, enabling them to reach the escaping prey with a few, but long strides. Their fore tibia are also provided with tibial pads, though poorly developed, suitable only to apprehend soft-bodied and slow-moving prey types. This is in contrast to the cylindrical, flattened or spatulate hairs on the well-developed Piratine and Ectrichodiine tibial pads (Haridass and Ananthakrishnan 1980b) which are efficiently used to grasp large, powerful prey types. The predators of these subfamilies are also characterised by their short powerful legs, short strides and fast running. The apical inner margin of the fore tibia of *R. marginatus* also possess tibial combs and this structure has already been reported in many Hemiptera (Breddin 1905; Davis 1969; Miller 1971; Swadner and Yonke 1973a; Yonke and Walker 1970; Weber 1930). These combs are used by the predators to groom the antennae immediately after the prey capture or after completion of feeding activity. The contention that the tibial pads are absent in *R. marginatus* and that the tibial combs of this insect facilitate efficient predation (Ambrose *et al* 1983) cannot be accepted, since it has been shown presently that there are tibial pads in the fore legs of *R. marginatus*, though in a poorly developed state and not efficiently used in prey capture as compared with the tibial pads of chase and pounce type reduviids (Haridass *et al* 1987), and that, as in many other reduviids (Swadner and Yonke 1973a), the tibial combs of *R. marginatus* are used only to clean the antennae from the foreign substances, like the body and salivary fluids from the attacked prey, either during or after predation. This grooming behaviour is significant since the antennae play important role in the predatory behaviour of reduviids.

The rostrum of *R. marginatus*, like other Harpactorinae, is long and slender and capable of considerable forward extension. This is aptly suited for the pin and jab type of feeding behaviour, where the rostrum and stylets are used to attack the soft-bodied prey types without the necessity to actively chase, to grab, to hold or to pounce on the prey as done by the chase and pounce type of reduviids. The latter reduviids are characterised by short curved rostrum capable of less than 45° forward extension, with which they probe for suitable site only after establishing a firm grip of the heavily sclerotised prey body.

Unlike other reduviids, *R. marginatus* and other soft prey feeding Harpactorine species do not often change their feeding sites. If the prey is held dangling at the rostral tip, feeding sites are never changed till the body content of the prey are fully emptied. The pin and jab type of feeding behaviour of *R. marginatus* as also true for many other Harpactorinae that feed on soft-bodied prey types, is unique and it is distinctly different from those of other subfamilies (Haridass and Ananthakrishnan 1980a; Haridass *et al* 1987).

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Ultrastructure of the eggs of Reduviidae: IV. Eggs of Rhaphidosomatinae (Insecta—Heteroptera)

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Abstract. Eggs of Rhaphidosomatinae share with the eggs of other subfamilies, several characters common to Reduviidae. The chorion with simple follicle cells, short, highly porous collar rim, 6 micropyles and the operculum with a central highly porous conical disc are characters to distinguish the eggs of this from other reduviid subfamilies. The ultrastructural details of the chorion, the operculum, and the aero-micropylar system of the eggs of *Rhaphidosoma atkinsoni*, the only species represented in India, are reported.

Keywords. Reduviidae; Rhaphidosomatinae; egg ultrastructure.

1. Introduction

The subfamily Rhaphidosomatinae is well represented in the Oriental and the Ethiopean regions, but *Rhaphidosoma atkinsoni* Bergrowth is the only species recorded from India so far (Distant 1977). Though the general description is provided by Readio (1926) and Miller (1953, 1971), the ultrastructural details of the eggs of this subfamily is yet to be worked out. Hence an attempt has been made on this aspect on the eggs of this species.

2. Materials and methods

Eggs of *R. atkinsoni* were collected, both from the field as well as from the laboratory reared insects. Methods presently used are similar to those described already (Haridass 1985b).

3. Observation

3.1 *Oviposition*

R. atkinsoni is found commonly among tall grass (*Aristida setacea* Retz. Poaceae). The gravid females glue cluster of eggs, singly or in twos or threes, on grass in a linear order and the eggs project obliquely from the long axis of the cylindrical grass stem, with the basal parts alone attached with the cementing materials (figure 1A). In one oviposition there may be as many as 17–22 eggs laid and a female lays 4–5 clutches of eggs in her life time, the number of eggs laid becoming decreased in subsequent ovipositions.

3.2 *Structure of the egg*

3.2a *Colour, shape and size*: Eggs of *R. atkinsoni* are light brown or straw coloured, but occassionly few of the eggs that fail to undergo tanning soon after
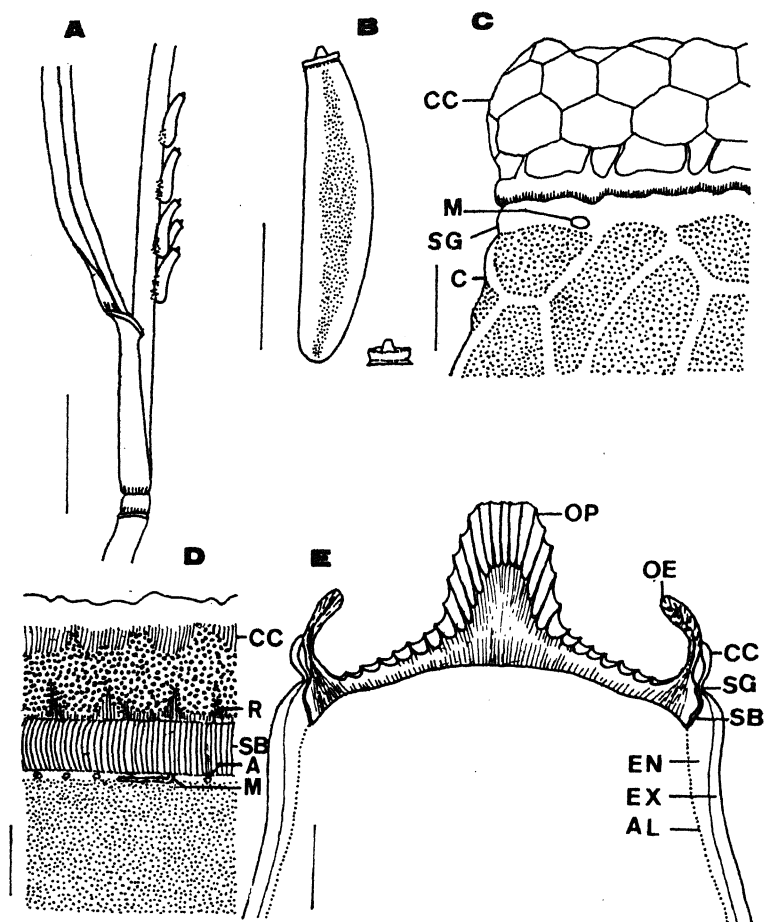
Figure 1. Eggs of *R. atkinsoni*. **A.** Egg mass attached to grass stem (scale = 5 m
B. Entire egg and operculum. Chorion shows lateral pigmented patch (scale = 1 m
C. Anterior part of entire egg (scale = 50 μ). **D.** Inner surface view of chorionic co
(scale = 50 μ). **E.** L.S. of the anterior half of the egg (scale = 50 μ).

oviposition, remain pale or light yellow. The lateral sides of the eggs show a lo
patch of dark brown pigments (figure 1B). The opercular region alone remains wh
The eggs are typically ellipsoidal and bottle-shaped, slightly concave on the ba
side (dorsal) and convex on the opposite (ventral/fore side). The posterior pole
pointedly rounded and the anterior pole is marked by the operculum with a co
like projection at the centre (figures 1B, E; 2A, G). The eggs measure 2
(± 0.002) mm in length from the opercular margin to the posterior pole and 0
(± 0.02) mm in width at the broadest point (n = 10).

3.2b *Body of the egg*: The main body of *R. atkinsoni* eggs is marked by hexago
follicular cells that originate from below the spermatic groove (figures 1C; 2B,
The boundaries of the cells are polygonal and thick near the neck region, but beco
regularly hexagonal and less prominent towards the posterior pole enclosing v
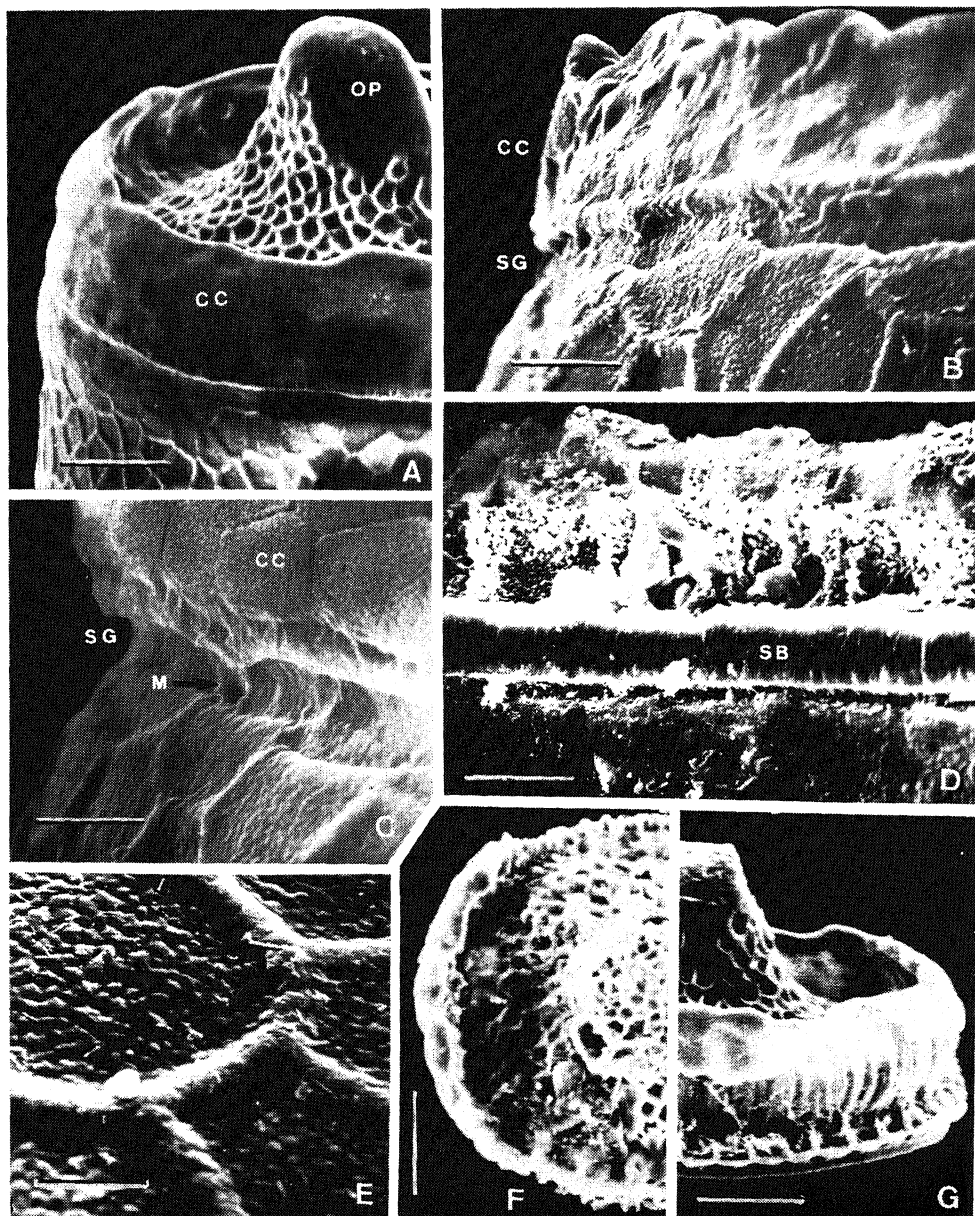
Figure 2. Scanning electron micrographs of *R. atkinsoni* egg. **A.** Anterior and opercular surface view (scale = 43 μ). **B.** Anterior part of egg after eclosion (scale = 10 μ). **C.** Micropylar opening in the spermatic groove (arrow) (scale = 7·5 μ). **D.** Inner view of chorionic collar without operculum (scale = 10·5 μ). **E.** Enlarged surface view of chorionic follicular cells (scale = 5·5 μ). **F.** Surface view of operculum (one half) (scale = 54 μ). **G.** Lateral view of operculum (one half) (scale = 42 μ).

(Abbreviations: A, Aeropyle inner opening; AL, aerostatic layer; C, chorion; CC, chorionic collar; EN, endochorion; EX, exochorion; OP, operculum; OE, opercular extension; M, micropyle; R, ridges on the inner surface of collar; SB, sealing bar; SG, spermatic groove).

shallow follicular pits, thus giving smooth appearance to the entire egg surface. ˙
follicular cells and its boundaries show minute warty projections (figure 2E).

The chorion is composed of outer exo- and inner endochorionic layers, the rela˙
thickness of the former being less than the latter in the collar region (exochorio˙
12·86 μ; endochorion = 14·29 μ); in the rest of the regions the exochorion is thic˙
(11·4 μ) than the endochorion (4·9 μ) (figure 1E). The faintly thickened follicu˙
boundaries are only extensions of the exochorion. Inner to the endochorion˙
present a network of aerostatic layer (1·43 μ), distributed uniformly throughout˙
interior of the egg and this layer originates from below the inner aeropylar openin˙
separated from the endochorion by small supporting struts. The aerostatic laye˙
distinctly absent in the opercular region (figure 1E).

3.2c   *Chorion and collar*:   The anterior end of the bottle-shaped eggs of *R. atkins*˙
is marked by a narrow neck or collar, consisting of an outer collar rim wholly m˙
up of exochorion and an inner sealing bar composed of endochorion. The ap˙
parts of the collar above the circular spermatic groove, continue as short, upwar˙
projecting continuous sheet of white and porous collar rim extensions (figures 1C˙
2A–D). The inner surfaces of these collar extensions (22·86 μ long) are supported˙
vertically tapering thick ridges extending from the base to the extremities (figure 2˙
The follicular markings of the chorion are very regular and contain network of˙
spaces (figures 1D; 2D). The inner basal parts of the collar rim extensions are uni˙
with the short lateral extensions from the opercular margins, thus affording f˙
anchorage to the latter (figure 1E). During eclosion, the opercular rim breaks at˙
fusion points between collar rim and opercular margins (figure 2G). The in˙
aspects of the collar rim facing the lumen of the egg exhibit many grooves and rid˙
that interlock with corresponding ridges and grooves of the operculum constitut˙
the sealing bar, with the help of which the operculum fits into the collar of the˙
(figures 1D; 2D, E).

3.2d   *Aeropyles and micropyles*:   The aero-micropylar system of *R. atkinsoni* is w˙
developed. The aeropyles continue as vertical canals from the spongy net-work of˙
spaces of the collar rim extensions and terminate as distinct circular openings at˙
origin of the inner aerostatic layer, just below the sealing bar (figure 1D). There ar˙
many as 58 aeropyles (SD ± 4). The micropylar number is always 6 in *R. atkins*˙
and the funnel shaped outer openings of the micropyles are located just above˙
lower limits of the shallow spermatic groove (figures 1C; 2C) and these also follo˙
course similar to that of aeropyles; but below the level of the sealing bar, they ma˙
'L' bend and terminate into the lumen of the egg (figure 1D) exhibiting a char˙
teristic clock-wise arrangement when viewed from the anterior pole of the egg.

3.2e   *Operculum*:   The operculum of *R. atkinsoni* resembles and fits into the co˙
rim like a lid of a jar, through the sealing bar composed of grooves and ridges. ˙
upper margin of the operculum inner to the points of union with the collar ext˙
sions, is produced into continuous, slanting extensions that appear club-shaped˙
cross-section, containing numerous pore canals leading through the solid opercu˙
body to finally open into the lumen of the egg (figure 1E). The centre of˙
operculum is produced into a conical flat-topped projection, also containing m˙
pore canals, that not only open outside at the flat surface, but also into the interio˙

the egg. The entire upper surface of the operculum is marked by polygonal honey-comb openings (figures 1E; 2F, G).

4. Discussion

The eggs of *R. atkinsoni* possess many typical characters like the operculum, chorion with exo- and endochorionic layers, a distinct collar containing outer spermatic groove and inner sealing bar, and a continuous inner aerostatic layer. Such characters have been reported in many reduviid subfamilies (Cobben 1968; Haridass 1985b, 1986a, b; Hinton 1981; Southwood 1956). Despite this, the eggs of Rhaphidosomatinae possess sufficient characters to distinguish these from the eggs of other reduviid subfamilies. The very shape of the egg, long, linear and bottle-shaped, with pointedly rounded posterior pole is a distinct feature, shared only by the eggs of Emesinae (Wygodzinsky 1966). In the method of oviposition, glueing the eggs to the substratum in an exposed manner, in the structure of the collar rim and in the origin and distribution of the aeropyles and micropyles, the eggs of *R. atkinsoni* resemble, among the various subfamilies of Reduviidae, only with those of Harpactorinae; but Rhaphidosomatine eggs can be distinguished from the latter by many other characters, particularly by the continuous sheet of short, spongy collar rim extensions as well as by the conical disc of the operculum containing numerous pore canals. These characters are typical for Rhaphidosomatinae and have been found in other species of this subfamily, including *R. circumvagus* Stal, *R. maximum* Miller, *R. griseum* Miller, *R. rufipes* Miller and *Lophodytes nigrecens* Miller (Miller 1953).

The opercular surface of *R. atkinsoni*, particularly the conical central disc, is provided with many pore canals traversing the thick opercular wall to open into the lumen of the egg. Their direct opening into the lumen compensates the absence of the aerostatic layer in the operculum. The chorionic collar rim extensions with their spongy air cavities and the aeropyles that originate from these air spaces provide air passage to the inner aerostatic layer. All these are specialised structures that enable the Rhaphidosomatine eggs to transport ambient oxygen to the interior of the egg.

The micropyles of *R. atkinsoni* is constantly 6 and this number appears to be the least for the micropyles in all the subfamilies examined so far (Haridass 1985b; 1986a, b) and this is well below the typical micropylar number suggested for the Reduviidae (Cobben 1968).

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Effect of two juvenile hormone analogues on embryonic morphogenesis, histogenesis, endocrines and cuticulogenesis of *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae)

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Abstract. The effect of topical application of juvenile hormone analogues farnesyl methyl ether and kinoprene (ZR 777) at different doses to eggs immediately after laying, germ band formation and blastokinesis, produced different types of abnormal embryos with varying degrees of derangement of development, most of them ultimately resulting in failure to hatch. Some of the embryos were almost normal but failed to hatch even though they continued to develop inside the chorion and died two days later. On the whole, there was correlation between dose of the analogue applied and mortality rate. Kinoprene was much more effective than farnesyl methyl ether. With given dose, per cent embryonic mortality was more or less the same whether the analogues were applied just after oviposition or germ band formation, but was lesser when applied immediately after blastokinesis. The period just after germ band formation appeared to be most sensitive. Treatments affected the endocrine system. The neurosecretory index was higher in the treated embryos. Prothoracic glands and their nuclei showed considerable enlargement in treated embryos continuing development inside chorion even after their controls hatched. The corpus allatum was smaller in treated embryos and corpora cardiaca were filled with neurosecretory material. Cuticle development was abnormal after treatment.

Keywords. *Dysdercus cingulatus*; juvenile hormone analogues; kinoprene (ZR 777); farnesyl methyl ether; embryonic development; morphogenesis; histogenesis; endocrines; cuticulogenesis.

1. Introduction

Ever since Slama and Williams (1966) reported that 'paper factor' affected embryonic development in *Pyrrhocoris apterus*, instances of juvenile hormone (JH) analogues leading to abnormalities in embryonic development have been reported in insects belonging to different groups and with different analogues (Slama 1974). The effects vary depending upon species, time of application, dosage applied, and the hormone analogue used. Dipteran embryos appear to be more resistant than the others and heteropteran embryos are very sensitive (Matolin 1971; Wright and Spates 1972; Enslee and Riddiford 1977; Patterson and Schwarz 1979). Some of our findings on the effects of the JH analogues farnesyl methyl ether (FME) and kinoprene (ZR 777) topically applied to eggs of *Dysdercus cingulatus* at different stages of development, on morphogenesis and on the endocrines (Jacob and Prabhu 1979a, b), ontogeny of the endocrine organs of the normal insect and their probable involvement in embryonic moulting (Jacob and Prabhu 1985), effects of the analogues at cellular level constituting inhibition of mitotic division and pycnosis of nuclei arresting mitotic activities at various stages of embryonic development (Jacob and Prabhu 1986b), and the significance of the endocrines and exogenous JH in embryonic development in *D. cingulatus* (Jacob and Prabhu 1986a) have been briefly reported.

The present paper deals in detail with the effect of JH analogues FME and Kino-prene on morphogenesis, histogenesis endocrine organs and cuticulogenesis in this insect.

2. Materials and methods

Rearing methods, collection of eggs of required age, and techniques employed have already been described (Jalaja and Prabhu 1976; Jacob and Prabhu 1985). The JH analogues used were, FME purchased from Eco Control Inc., USA and Kinoprene (ZR 777) a gift from Dr G B Staal of Zoecon Corporation, USA. The analogues were dissolved in acetone, and 1 μl containing the required dose were topically applied by means of a microliter syringe, to eggs (i) at oviposition; (ii) immediately after germ band formation and (iii) just after blastokinesis. Acetone treated eggs served as controls. Each group consisted of 25 eggs, and the experiments were replicated several times to get sufficient number of embryos for the study. Their development was followed, by fixing and processing them as described for normal embryos (Jacob and Prabhu 1985).

3. Results and discussion

3.1 *Abnormal embryos*

FME and kinoprene (ZR 777) applied to eggs of *D. cingulatus* at different stages and doses resulted in different types of abnormal embryos. Figures 1–6 are the photographs of their whole mount preparations and figures 7–13 give their histological picture. Some of the embryos are comparable to those reported in *Schistocerca gregaria* (Novak 1969); some are comparable to those in *Pyrrhocoris apterus* (Matolin 1971) and some others resemble those reported in *Pyrrhocoris* by Ensley and Riddiford (1977); some are new abnormalities not reported earlier in any species. These abnormalities and their appearance and histological picture in *D. cingulatus* are described.

3.1a *Non-segmented embryo*: The non-segmented embryo was the most severe abnormality and showed maximum derangement of development. In whole mount it appeared as red spot at the posterior pole of the egg (figure 1). However, the structure of those embryos varied, on the basis of which these may be divided into two:

(i) *Undifferentiated embryo*: This type of embryo was made up of disorganised mass of cells without differentiation into tissues or organs. Disintegrating nuclei were also observed in them. Blastoderm cells appeared to show no mitosis and this embryo did not proceed to form germ band.

(ii) *Non-segmented embryo with rudiments of appendages*: This type of embryo was divisible into a small anterior part, the head, and a tapering part together constituting the thorax and the abdomen. The embryo was only half the size of the egg. There were two or three appendage rudiments; those faced the yolk and hence were not visible in whole mounts. Appendage rudiments were not further divisible. A small brain and 3 thoracic ganglia could be observed in this embryo. The abdominal
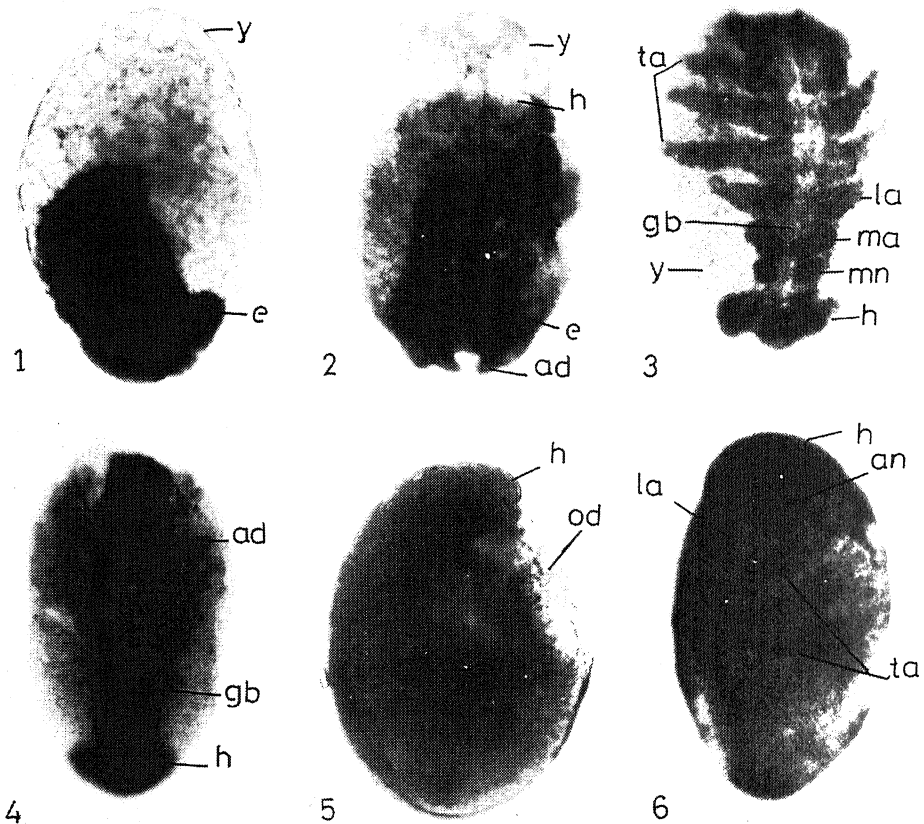
Figures 1–6. Whole mounts of embryos; Feulgen method. **1.** Non-segmented embryo (× 100). **2.** Dwarf embryo (× 100). **3.** Fully segmented germ band (× 100). **4.** Deformed germ band (× 100). **5.** Embryo without dorsal closure (× 100). **6.** Embryo with short and stumpy appendages (× 100).

region was represented by a few scattered cells most of which were big having pycnotic nuclei (figure 7). Some of these embryos showed a germ band with developing anterior region while the blunt end of the germ band did not show any differentiation. Masses of cells seemed to sink into the yolk from the anterior part of the germ band. The amnion cells appeared more spherical. It appears that the embryo remained at the posterior pole of the egg without undergoing blastokinesis.

3.1b *Dwarf embryo*: Though this showed marked differentiation (figure 2), it did not hatch and was quite common. The body was divisible into head, thorax and abdomen. All the appendages were present. The embryo was usually seen in the posterior pole or at the middle region of the egg. The embryo got pigmented on fourth day, the pigmentation deepened on fifth day and red eye spots developed. Till the normal time of hatching of controls, this embryo showed movements inside the chorion but it failed to hatch. A well-formed nervous system occupied the major part of the body. The gut was highly reduced. Neither muscle fibres nor gonadal rudiments got differentiated. When the JH treated eggs were fixed on third day, some of them

appeared with completed germ band but the germ band and the appendages were shorter compared to controls (figures 3 and 4). Masses of undifferentiated cells could be seen in the inner layer.

3.1c *Embryo without dorsal closure*: This appeared frequently after JH treatment. The size of the embryo varied. It had no dorsal ectoderm and the gut was in direct contact with the yolk. Body was divisible into head, thorax and abdomen. All body segments and appendages were present irrespective of the size of the embryo. Two
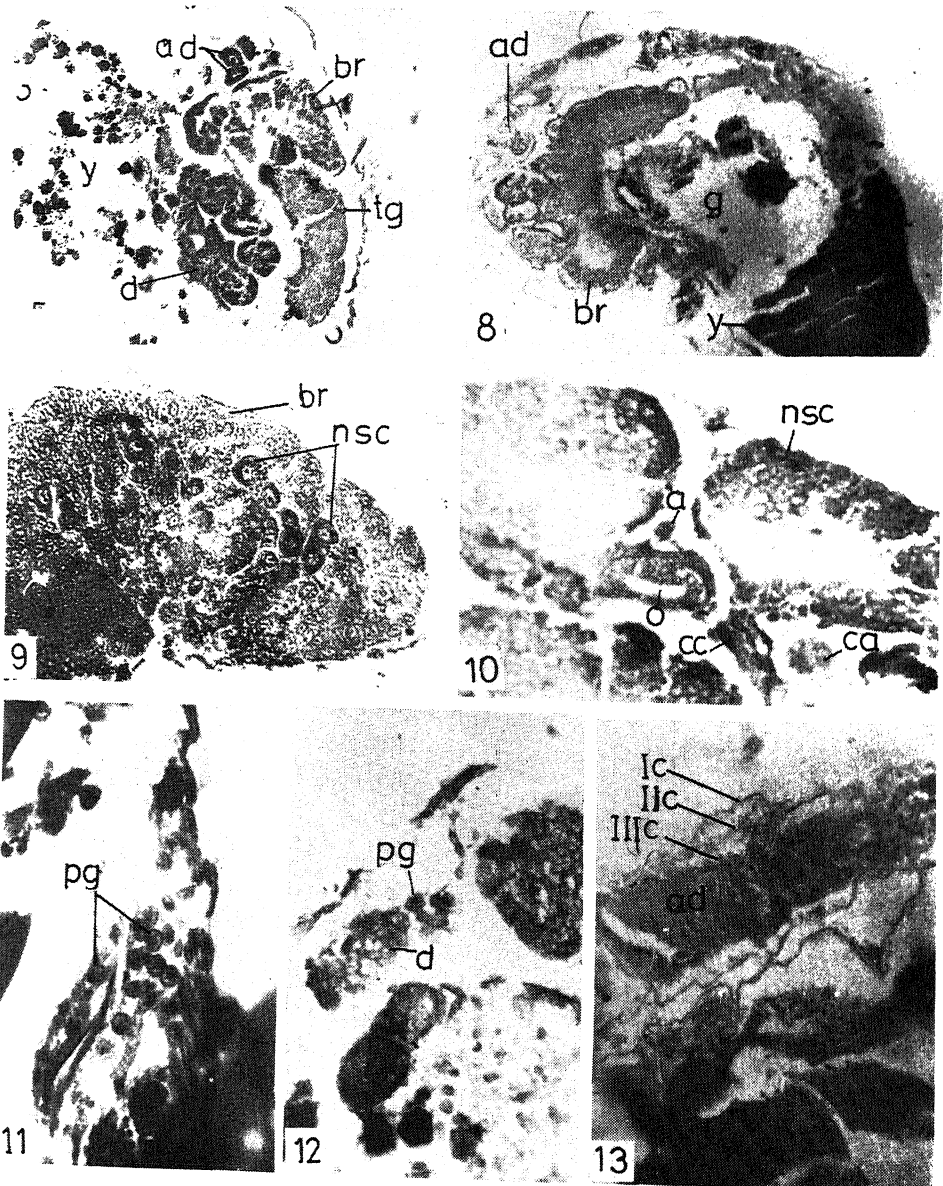
Figures 7–13.

types of embryos were identifiable. In the first group, there was dorsal ectoderm which was however, incomplete. So the central portion of thorax and abdomen remained open (figure 5). This abnormal embryo got pigmented except at the open area. It had red eye spots, feeble muscle fibres and differentiated gonadal rudiments; the embryos got pigmented on fourth day. In the second type, the abnormality was more severe. It showed no sign of development of a dorsal ectoderm. The dorsal side remained without pigmentation.

3.1d *Embryo with sunken head*: This type of embryo was observed only after treatment with kinoprene. The head was not visible on the surface. The appendages appeared to originate from the dorsal side of the body. A number of foldings of ectoderm were observed at the thoracic region. This embryo was smaller. It got pigmented and body movements were observed till the hatching time of controls. The brain was shifted to about half the way through the dorsal side of the body. The central nervous system was smaller. The dorsal ectoderm was transparent; the gut was reduced in size. Muscle fibres were present on the ventral side of abdominal segments. Genital rudiments could not be identified in this embryo. Masses of undifferentiated cells could be seen in the abdominal region (figure 8).

3.1e *Embryo with short and stumpy appendages*: This was rather rare among the unhatched eggs. In this, none of the body segments were affected, though normal number of segments were lacking in the appendages (figure 6) Pigmentation and size was normal. Nervous system did not consolidate. The gut was enlarged occupying the major part of the embryo.

3.1f *Less pigmented embryo*: Normally, the embryos had light yellow pigmentation on fourth day which changed to an orange red shade before hatching. However, some of the treated embryos remained colourless while in others the light yellow pigmentation remained till fifth day. The eye pigmentation also was reduced considerably. The nervous system got consolidated but in most of the embryos the

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Figures 7–13. Sections of embryos. **7.** Sagittal section through non-segmented embryo with rudiments of appendages. The differentiated nervous system is found in the midst of undifferentiated tissues (× 100) (Smith's fluid, Heidenhain's Haematoxylin eosin). **8.** Sagittal section through embryos with sunken head (× 100) (Smith's fluid, Heidenhain's Haematoxylin eosin). **9.** Sagittal section through brain of dwarf embryo showing neurosecretory cells (× 400) (Smith's fluid, Gomori's Chrom haematoxylin phloxin). **10.** Horizontal section through head of less pigmented embryo. Brain neurosecretory cells, corpus cardiacum, corpus allatum and aorta are filled with secretory material (× 400) (Smith's fluid, Gomori's Chrom haematoxylin phloxin). **11.** Sagittal section through an embryo which completed development but failed to hatch showing prothoracic glands (× 400) (Smith's fluid, Gomori's Chrom haematoxylin phloxin). **12.** Sagittal section through embryos which continued development inside the chorion even after hatching time showing enlarged prothoracic gland cells (× 400) (Smith's fluid, Gomori's Chrom haematoxylin phloxin). **13.** Sagittal section through dwarf embryo showing abnormal cuticle deposition around appendages (× 600) (Smith's fluid, Heidenhain's haematoxylin eosin).

(Abbreviations: a, Aorta; ad, appendage; an, antenna; br, brain; Ic, first cuticle; IIc, second cuticle; IIIc, third cuticle; ca, corpus allatum; cc, corpus cardiacum; d, degenerating cells; e, embryo; g, gut; gb, germ band; h, head; la, labium; ma, maxillary appendage; mn, mandibular appendage; nsc, neurosecretory cell; o, oesophagus; od, open dorsal side; pg, prothoracic gland; ta, thoracic appendage; tg, thoracic ganglia; y, yolk).

neurons had no compact arrangement. Muscle fibres were distinguishable but n[...]
gonads differentiated. Masses of disintegrating cells were seen in the abdomen.

3.1g *Embryo which completed development but failed to hatch*: The most commo[...]
abnormality was that which resulted in failure of apparently normal embryo t[...]
hatch. Till the hatching time the embryo showed movements inside the chorion; i[...]
got pigmented normally but the movements stopped a few minutes after norma[...]
hatching time of controls. Histological studies however revealed disintegrating nucle[...]
throughout. All systems got differentiated but many nuclei were pycnotic.

3.1h *Embryos which died while hatching*: These constituted the embryos whic[...]
apparently not only completed development but also made attempts and struggle[...]
to come out of the chorion, but failed to hatch. The appendages were wrapped in [...]
transparent membrane. There were fewer disintegrating nuclei in them.

3.1i *Embryo continuing development inside the chorion even after hatching time[...]*
Among the unhatched eggs, some continued development further, though rarely[...]
These embryos showed movements till two days after normal hatching time of con[...]
trols. Pigmentation changed to red like those of a two day old first instar nymph[...]
These embryos were enclosed in a transparent membrane inside the chorion[...]
Nervous system got consolidated; mesoderm derivatives were present, and the siz[...]
was also normal.

It may be seen that embryos with sunken head have not been reported elsewhere[...]
embryos with lesser pigmentation has been reported only in *Thermobia* (Matolin an[...]
Rohdendorf 1972). We have not noted any twin embryo as reported in *Thermobia* b[...]
Rohdendorf and Sehnal (1973).

3.2 *Hatchability studies*

There was clear correlation between dose of the analogue applied and mortality of th[...]
embryos including all the abnormalities produced ultimately resulting in failure t[...]
hatch, when either of the analogues were applied. However, 0·25 and 0·5 μg FMI[...]
had little effect. Generally, the undifferentiated mass of cells referred to as non[...]
segmented embryos, was the most severe abnormality. Kinoprene was much mor[...]
effective than FME. The former was over 100 times active, its LD_{50} is 1/125 of th[...]
latter. With given dose, per cent embryonic mortality was more or less the sam[...]
whether the analogues were applied immediately after oviposition or germ band[...]
formation, there being no difference in mortality. However, the effect was less whe[...]
applied immediately after blastokinesis. Germ band stage was the most critica[...]
sensitive period. However, with regard to the type of abnormality produced, ther[...]
was considerable difference. In this respect the present findings on *D. cingulatus* ar[...]
different from those on *Hyalophora cecropia* (Riddiford 1971) where the effect wa[...]
delayed due to the presence in the eggs of longer lasting stored material gen[...]
products.

Mitotic inhibition and nuclear pycnosis and degeneration occurring especially a[...]
germ band formation (Jacob and Prabhu 1986b) are the causative factors o[...]
abnormalities resulting in embryos made up of undifferentiated mass of cells. Where[...]
severity of treatment was less, germ band formation continued, but blastokinesi[...]

failed to occur; where the effect was least, blastokinesis also continued but hatching was prevented. Most of the embryos died at this point but some which struggled to hatch, continued development for some time till they succumbed to death. Endocrines were also affected, especially prothoracic gland activity; so cuticulogenesis and moulting were disrupted. It is clear from the studies that though corpus allatum was inactive during embryonic development in *D. cingulatus* (Jacob and Prabhu 1985), exogenous JH affects embryonic development decisively.

3.3 Endocrine system

The data on the neurosecretory system is analysed and presented in table 1. It may be seen that neurosecretory index is lower in control insects whereas in treated ones resulting in abnormal development, the index is comparatively high, the greater the abnormality, the higher the neurosecretory index. Thus, the embryos which died while hatching, had almost the same index as in the controls whereas non-segmented as well as dwarf embryos (figure 9) had many times the index compared to that of the controls at the time of hatching. Neurosecretory cell volume and nuclear volume of embryos with drastic abnormalities, were very high, though in many other types of abnormal embryos their values were slightly less than in the controls. Prothoracic gland cells and their nuclei were considerably enlarged in treated embryos which continued development inside chorion even after normal hatching time (figure 11). In other abnormal embryos they were more or less comparable to controls (table 1). Corpus allatum (table 1) was comparatively smaller in treated embryos except in those which continued development inside chorion. This could be due to a negative feed back action of exogenous analogue applied. Though during normal development corpus allatum did not show much evidence of activity as indicated by increase of size (Jacob and Prabhu 1985) the present finding of feedback inhibition of exogenous JH analogues in treated embryos showed that they were active at least in 5 day embryos. Corpus allatum of the treated individual could not fully compensate for the excess titre by inhibiting its own activity and abnormality results from the resultant upset of hormonal milieu. Treatment after blastokinesis produced lesser effect evidently because by then corpus allatum developed and started its activity and its own shutting off of activity to a certain extent regulated the hormone titre. It may be noted that though juvenile hormones and ecdysteroids have been reported in eggs and embryos of insects (Hoffmann and Lagueux 1985) there is very little evidence that juvenile hormone is indeed synthesized in the embryo corpus allatum. The present finding of a feedback inhibition of allatum in treated embryos shows that the corpus allatum is active at least in 5 day old embryos though it was not possible to observe any size increase of corpus allatum in our earlier studies (Jacob and Prabhu 1985). Evidence of capability of embryos for JH synthesis has been obtained by Bergot *et al* (1981) in *Manduca sexta* and the present findings localises this site at corpus allatum, in *D. cingulatus*. Unlike in the case of corpus allatum, it has been possible to demonstrate active synthesis by embryonic prothoracic glands, of ecdysteroids; our earlier findings (Jacob and Prabhu 1985) suggest activity of the glands in the embryo in *D. cingulatus,* and the present work shows exaggerated size increase of prothoracic gland cells and their nuclei after exogenous treatment of JH especially in embryos which continued development inside chorion even after hatching time, implicating greater activity of the prothoracic glands in these treated

Table 1. Changes in the neurosecretory cells, prothoracic gland cells and corpus allatum of 5 day old abnormal embryos resulting from JH treatment (Mean ±SD).

Type of embryo	Neurosecretory cells				Prothoracic gland			Corpus allatum		
	Cell volume (μm^3)	No.	Index	Nuclear volume (μm^3)	Cell volume (μm^3)	Nuclear volume (μm^3)	Volume of Ca (μm^3)	No. of nuclei	Mean cell volume (μm^3)	Nuclear volume (μm^3)
Non segmented embryo	1554·09±213·89 (702·90±28·19)	17 (3)	20	423·25±52·42 (301·01±8·60)	Prothoracic gland not differentiated		Corpus allatum	Not differentiated		
Dwarf embryo	1660·12±315·55 (698·90±67·62)	16 (3)	19	403·50±157·65 (291·11±9·11)	49·64±1·55	6·62±0·31	1304·20±117·42	28±2·11	44·60±0·43	4·18±2·16
Embryos without dorsal closure	599·46±12·02 (159·44±6·28)	10 (4)	14	178·08±23·59 (60·03±3·98)	66·63±2·58	8·18±0·12	2618±44·20	48±4·12	52·98±0·81	6·62±1·01
Embryos with sunken head	143·75±7·33 (93·28±4·11)	9 (4)	13	32·64±1·69 (22·54±4·47)	71·89±1·04	1·49±0·48	2054±14·11	40±2·24	51·25±0·31	5·40±0·11
Embryos with short and stumpy appendages	130·25±24·33 (103·24±14·09)	10 (5)	15	19·72±3·28 (16·01±2·27)	78·97±1·35	6·62±0·34	2628·40±16·36	46±2·11	57·90±1·13	6·62±1·11
Embryos with less pigmentation	324·87±10·80 (117·84±8·14)	11 (3)	14	83·54±3·03 (41·76±4·46)	65·44±1·23	5·57±0·29	2600±20·20	49±3·31	52·98±1·18	6·62±2·10
Embryos which completed development but failed to hatch	169·40±13·09 (102·51±19·61)	6 (2)	8	25·17±5·91 (14·13±1·52)	47·71±1·35	6·78±0·67	2675±12·16	52±2·38	51·25±1·50	5·40±2·16
Embryos which died while hatching	164·31±10·09 (105·78±12·80)	5 (2)	7	20·11±1·76 (17·67±1·24)	46·45±0·70	4·84±0·77	2760±8·40	54±2·81	51·25±1·50	6·62±2·81
Embryos which continued development inside the chorion	279·39±81·35 (153·75±22·42)	6 (3)	9	57·67±8·19 (49·38±5·72)	381·70±3·52	33·51±1·65	3470±31·11	60±3·31	57·90±1·81	6·92±3·11
Control	343·70±29·18 (173·51±10·91)	3 (2)	5	63·18±2·61 (31·61±2·17)	73·62±1·55	5·27±0·11	3590±115·02	62±6·12	57·90±5·11	6·92±2·36

embryos. On the whole the embryos which continued development inside the chorion are the least affected ones. Their greater prothoracic gland activity was indicative of some problem with moulting. The most affected ones are the non-segmented embryos, dwarf embryos and those with dorsal closure, as it was before corpus allatum developed and hence could not regulate the hormone titre by regulating its own production.

Additional noteworthy features of the changes in the endocrine system after treatment are, that the corpora allata, corpora cardiaca and the prothoracic glands were not differentiated in the non-segmented embryos. The corpora cardiaca were filled with secretory material in almost all treated, abnormal embryos (figure 10); the corpora allata showed no cyclic activity in which respect it was comparable to that of the controls; however, they were smaller in size in treated individuals. The prothoracic glands had considerable pycnotic nuclei, fewer cells showing cyclic activity (figure 12). Exceptions were those embryos which continued development inside the chorion; fewer nuclei were pycnotic in them and their nuclei had increased in size considerably.

3.4 *Cuticulogenesis*

No cuticle was found in non-segmented embryos. However, in the dwarf embryos there were 3 cuticles covering the appendages (figure 13) whereas only one cuticle covered the dorsal and ventral aspects of the body. In the embryos without dorsal closure, two embryonic cuticles were observed around the appendages and ventral aspects of the body. These appeared to represent the first and the second cuticles as they were very thin, the thicker third cuticle attached to the epidermis. Only one moult has taken place in these abnormal embryos. The embryos with sunken head had only one cuticle almost throughout the body. At the head region however, thick cuticle was evident. Around the head and appendages of the embryo with short and stumpy appendages, two cuticles were clear. They represented the second and third cuticles, as the cuticle attached to the epidermis was thicker. Only one cuticle was seen in the ventral and dorsal aspects of the body. Less pigmented embryos had only one cuticle covering the body. This cuticle got detached at different regions of the dorsal and ventral sides but no new cuticle was seen beneath the detached cuticle. In these embryos only the first cuticle was initiated and even that was not complete. In the embryos which completed development but failed to hatch, those which died while hatching and those which continued development inside the chorion even after hatching, had a transparent membrane covering the embryos when the chorion is removed. In histological preparations this membrane could be identified as the composite of two cuticles which are closely packed together. They got detached from the embryo. In addition, there was a thick, third cuticle attached to the epidermis. Thus these embryos had all the 3 cuticles characteristic of the normal embryo. Two abortive moults occurred in these embryos, though they failed to hatch ultimately. Hatching was prevented due to the presence of this membrane constituting the composite first and second cuticles. This situation is reminiscent of the condition in *Spodoptera littoralis* where excess juvenoids entail blockage of metamorphosis, the treated larvae continuing to grow until the juvenoids disappear, never moulting till they perish even after 30 days (Gelbic and Sehnal 1986). Hyperactivity of the prothoracic glands as indicated by the larger size of the cells and the nuclei observed in

the present study is an attempt on the part of the prothoracic glands to circum
the excess JH analogue present in the embryo. It may be noted that coinciding
embryonic moults, balanced high peaks of ecdysone and juvenile hormone have b
reported (Imboden *et al* 1978) which are necessary for embryonic cuticulogenesis
moulting. Exogenous juvenile hormones are also known to inhibit ecdyster
synthesis not only in insects like *Blattella germanica* (Masner *et al* 1975) bu
embryos as well, as in *Rhodnius prolixus* (Patterson and Schwarz 1979). In *Oncop*
fasciatus embryos the source of intrinsic ecdysteroid production is thought to
oenocytes (Dorn and Romer 1976) whereas in *D. cingulatus* embryos the pre
study confirm our earlier suggestion that prothoracic glands appear to be involve
ecdysteroid synthesis (Jacob and Prabhu 1985).

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Digestive enzyme secretion during metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. Protease, amylase, lipase and trehalase are present in the larval and adult midguts of *Oryctes rhinoceros*; cellulase is absent. Invertase is present only in the adult. The presence of trehalase in the pupal midgut suggests that food digestion is not the normal function of gut trehalase. Quantitative studies reveal that protease and amylase in the third instar larvae reach very low levels as the larvae become older and consume little or no food. These two enzymes are not measurable in the non-feeding prepupa and pupa and reappear in the adult with commencement of feeding. Secretion of digestive enzymes is correlated with feeding.

Keywords. Coconut rhinoceros beetle; *Oryctes rhinoceros*; metamorphosis; digestive enzymes.

1. Introduction

Factors regulating the secretion of digestive enzymes in insects have recently been reviewed by Chapman (1985). In most of the insects studied, changes in digestive enzyme secretion occurred in relation to food intake (Dadd 1956; Baker 1976; Muraleedharan and Prabhu 1978). However, in a few instances digestive enzymes were noticed even in the absence of feeding (Dadd 1956; Langley 1967). Secretion of digestive enzymes also changed during metamorphosis reflecting their changing feeding habits (Spiro-kern 1974; Eguchi and Iwamoto 1975). The larva and the adult of *Oryctes rhinoceros* exhibit different feeding habits. While the larval food consists of cattle dung and decaying coconut stumps, the adult feeds on juice obtained from the tender parts of the coconut palm. Being a holometabolous insect, the animal possesses a non-feeding pupal stage in its life cycle. The animal also attains considerable size during the larval period, the third (final) instar gaining a weight of about 10 g and hence is a suitable insect for study. It was, therefore, thought worthwhile to investigate the changes in digestive enzyme secretion during metamorphosis of this animal.

2. Materials and methods

The larvae, prepupae and pupae of *O. rhinoceros* used for the study were taken from the stock colony reared on sterilised cowdung as described by Mini and Prabhu (1986). Adults were maintained on slices of ripe banana (Kannan and Prabhu 1985).

2.1 *Preparation of the enzyme extract*

The method of Applebaum *et al* (1964) was followed for the preparation of the

midgut extract. The concentration of the homogenate was adjusted to 1 midgut/5 ml distilled water.

2.2 *Qualitative study of digestive enzymes*

The presence or absence of amylase, invertase, trehalase and cellulase was studied by the method of Noelting and Bernfeld (1948) with some modifications. The reaction mixture contained 0·2 ml Tris-HCl buffer (pH 8·2), 0·2 ml enzyme extract and 0·4 ml 1% substrate solution (table 1) and it was incubated for 2 h. Lipase activity was studied by the method of Rockstein and Kamal (1954) using a reaction mixture containing 0·2 ml coconut oil, 0·2 ml buffer (pH 7) and 0·4 ml enzyme extract. Incubation was carried out for 4 h.

2.3 *Quantitative estimation of digestive enzymes*

Amylase and protease were studied quantitatively with a spectrophotometer. Amylase activity was estimated using the method of Noelting and Bernfeld (1948) limiting the incubation time to 20 min. The procedure of Birk *et al* (1962) was followed for estimating protease activity. The reaction mixture had a composition as described for amylase. Glycine-NaOH buffer (pH 9) was used and incubation period was 30 min.

2.4 *Control*

Enzymes denatured by heating the midgut extract at 100°C for 10 min served as control in all experiments.

2.5 *Measurement of food taken by larva*

Weight of larva together with excreta after feeding for 24 h minus weight of larva before feeding was taken as the amount of food consumed by the larva per day (Langley 1966).

3. Results

Data on qualitative studies on digestive enzymes are summarised in table 1. The per day consumption of food by the third instar larva increased with age. The larvae as

Table 1. Digestive enzyme activity during metamorphosis in *O. rhinoceros*.

Enzyme	Substrate used	Larva	Pupa	Adult
Protease	Casein	+ + +	−	+ + +
Amylase	Starch	+ + +	−	+ + +
Lipase	Coconut oil	+ +	−	+ +
Invertase	Sucrose	−	−	+
Cellulase	Carboxymethyl cellulose	−	−	−
Trehalase	Trehalose	+ +	+ +	+ +

+ + +, High activity; + +, medium activity; +, low activity; −, no activity.

they became older consumed little or no food and started losing weight. In about 20–30 days time, they became considerably reduced in size and weight. Evacuation of most of the midgut contents occurred at this stage and the midgut contained no fresh food material. The midguts of the prepupa and pupa were completely devoid of any food material. Results of quantitative studies on digestive enzymes are given in table 2. Secretion of protease and amylase reached a maximum in the 14–16 week old larva and then declined to low levels. These two enzymes were absent in the prepupa and the pupa and reappeared in the adult with commencement of feeding.

4. Discussion

Digestive enzymes were studied in the larva of *Oryctes nasicornis* using only qualitative methods (Bayon 1980). Reported here are the results of both qualitative and quantitative studies on metamorphic changes in digestive enzyme secretion in *O. rhinoceros*. The results of qualitative studies revealed that the larva and the adult of this insect possess same digestive enzymes except invertase which is present only in the adult. This is due to the fact that unlike the larva, the adult feeds on fresh plant material which contains a good amount of sugars (Dadd 1985). Although cellulase was not observed in any of the stages in *O. rhinoceros*, the possibility of cellulose digestion in the larva by gut micro-organisms cannot be ruled out since the food of scarabacidae larvae contains cellulose which forms an important source of energy (Bayon 1980). In the larva of *O. nasicornis* no cellulase activity was demonstrated in gut contents or epithelium (Bayon 1980); but detection of carbohydrate fermentation products such as volatile fatty acids and methane provided evidence for microbial cellulolysis (Bayon 1980; Bayon and Mathelin 1980). The pupa of *O. rhinoceros* being a non-feeding stage, has lost its digestive capacity as evidenced by the closure of the

Table 2. Body weight, food consumed and secretion of protease and amylase at various stages of *O. rhinoceros*.

Stage	Body weight (g)	Food consumed (g)	Protease units[a]/ midgut	Amylase units[b]/ midgut
Third instar 3–4 weeks old	8·61 ± 0·39 (9)	2·54 ± 0·32 (9)	95·62 ± 3·38 (7)	260·47 ± 8·89 (7)
Third instar 7–9 week old	10·86 ± 0·49 (10)	4·51 ± 0·23 (10)	122·66 ± 8·95 (8)	454·51 ± 11·85 (8)
Third instar 14–16 week old	13·87 ± 0·38 (9)	4·61 ± 0·46 (9)	140·82 ± 11·66 (9)	482·32 ± 9·66 (9)
Third instar older larva	10·67 ± 0·45 (8)	NC	20·69 ± 8·46 (8)	45·99 ± 24·11 (8)
Prepupa	NC	Non-feeding	Not measurable	Not measurable
Pupa	NC	Non-feeding	Not measurable	Not measurable
Adult, female	NC	NC	73·03 ± 25·79 (7)	385·48 ± 72·38 (7)
Adult, male	NC	NC	56·82 ± 32·24 (8)	237·65 ± 37·13 (8)

[a] One unit = the amount of enzyme required to liberate one μg of tyrosine per min.
[b] One unit = the amount of enzyme required to liberate one μg of maltose equivalents per min.
NC, Not calculated.
Number of determinations given in brackets.

mouth and anal openings and the progressive histomorphological transformations of the gut during the pupal period (unpublished results). Therefore, the presence of trehalase in the pupal midgut of *O. rhinoceros* as in *Bombyx mori* (Sumida and Yamashita 1977) suggests that food digestion is not the normal function of gut trehalase. The results suggest that qualitative changes in digestive enzyme secretion during metamorphosis in *O. rhinoceros* are in accordance with different feeding habits of the larva and the adult. The lepidopterans are a good example in this respect (House 1974).

Feeding is found to influence the secretion of digestive enzymes in *Dysdercus cingulatus* (Muraleedharan and Prabhu 1978). In the older larvae of *Attagenus megatoma* a decline in protease secretion was noticed which was attributed to a decreased intake of food (Baker 1976). Even feeding damp cellulose powder or water resulted in increased secretion of protease in adults of *Tenebrio molitor* (Dadd 1956). Furthermore, protease activity was not observed in the midgut of prepupa (Dadd 1956) and pupa (Langley 1967; Baker 1976). In the present study maximum protease and amylase secretions were observed in the 14–16 week old larva of *O. rhinoceros* which consumed the maximum amount of food. The midguts of older larvae contained no fresh food material. Secretion of protease and amylase reached very low levels at this stage. The absence of protease and amylase in the non-feeding prepupa and pupa is correlated with lack of feeding. These two enzymes reappeared in the adult with commencement of feeding. It may be seen that secretion of protease and amylase in different stages of *O. rhinoceros* shows considerable changes in relation to the state of feeding. Thus, from this study it appears that qualitative changes in digestive enzyme secretion during metamorphosis in *O. rhinoceros* occur as a result of transition from larval feeding habit to the adult type. Regarding the quantity of digestive enzymes present in various stages of development, secretion of digestive enzymes is correlated with feeding.

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Probable endocrine role of midgut tissue in stimulation of digestive enzyme secretion in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. The effect of midgut epithelial extract on digestive enzyme secretion in the third instar larva of *Oryctes rhinoceros* was studied. Midguts dissected out from third instar larvae were cut at both ends, emptied of their contents, washed in insect saline, ligated at the open ends and incubated in a medium containing extract of 2 midgut epithelia/10 ml incubation solution. In control experiments midgut preparations were incubated in insect saline without midgut epithelial extract. After 30 min of incubation, the contents of the midgut preparations incubated with midgut epithelial extract showed increased secretion of protease and amylase when compared with controls. Digestive enzyme release into gut lumen appears to be due to the action of a hormone present in the midgut epithelium.

Keywords. Coconut rhinoceros beetle; *Oryctes rhinoceros*; digestive enzymes; gut hormone.

1. Introduction

Secretagogue, neural and endocrine mechanisms have been variously postulated in different insects in the regulation of digestive enzyme secretion (House 1974). Reports on endocrine mechanism mostly deal with the role of the brain median neurosecretory cells in the secretion of digestive enzymes (Briegel and Lea 1979; Muraleedharan and Prabhu 1979). By injecting midgut extracts into haemocoel of the cockroach *Periplaneta americana*, Rounds (1968) found that a substance in the midgut epithelium which stimulated digestive enzyme secretion could act as a possible intermediate link in the regulatory mechanism between the central nervous system and the midgut tissue. Recent ultrastructural studies have shown the presence of endocrine cells in the midgut epithelium of insects (Endo and Nishiitsutsuji-Uwo 1981; Nishiitsutsuji-Uwo and Endo 1981). Immunological techniques have, meanwhile, provided additional evidence for the occurrence of several vertebrate-like gut peptides in the insect gut epithelium (Raabe 1983). These findings indicate that in insects the midgut itself can be a source of hormonal principles regulating digestive enzyme secretion. However, this possibility is yet to be conclusively demonstrated. The *in vivo* method of Rounds (1968) referred to above has the drawback that the results obtained could as well be secondary, the immediate action of the midgut epithelial extract being on other target organs. Hence, an *in vitro* method was devised to find out the effectiveness of extracts of midgut epithelia on digestive enzyme secretion in *Oryctes rhinoceros* which is a highly suitable animal for the study (Sreekumar and Prabhu 1988). The findings have been briefly reported elsewhere (Sreekumar and Prabhu 1987) and the present paper is a detailed report on the findings.

2. Material and methods

Third (final) instar larvae of *O. rhinoceros* reared in the stock colony as described by Mini and Prabhu (1986) but on sterilised cowdung, were used for the study.

2.1 *Preparation of midgut epithelial extract*

Midguts from third instar larvae of *O. rhinoceros* were dissected out in insect saline (Starratt and Steele 1980). The gut contents were removed by opening the midgut and washing the tissues in several changes of insect saline. The tissues were homogenised in ice-cold insect saline. To remove protease and amylase from the homogenate, 50 mg casein and 50 mg starch were added to the solution. The homogenate was first filtered through glass wool and then centrifuged at 10,000 g for 10 min. The concentration of the homogenate was adjusted to 2 midgut epithelia/ 10 ml insect saline.

2.2 *Preparation of midgut for incubation*

Midguts dissected out from third instar larvae were cut at both ends and gut contents were removed. The midguts were then washed thoroughly in 5–6 changes of insect saline. Next, the two ends of the midgut were ligated using hair. The entire procedure took less than 10 min.

2.3 *Bioassay*

A glass tube of 6×2.4 cm internal diameter, open at both ends, one end of which was fitted with a rubber stopper through which a hypodermic needle was inserted served as the incubation chamber. Oxygen was delivered through the hypodermic needle. The bioassay apparatus was kept in a water bath at 37°C. The incubation chamber of the bioassay apparatus contained 10 ml midgut epithelial extract. The midgut preparation was suspended in it and incubated for 30 min under a small stream of oxygen bubble. After incubation, the midgut preparation was taken out, washed in 3–4 changes of insect saline and opened and the contents were collected in 1 ml ice-cold distilled water for quantitative estimation of protease and amylase.

2.4 *Quantitative determination of digestive enzymes*

The procedure of Birk *et al* (1962) with some modifications was followed for the determination of protease activity. The reaction mixture consisted of 0.2 ml enzyme extract, 0.2 ml glycine-NaOH buffer (pH 9) and 0.4 ml 1% casein solution. Enzyme activity was terminated after 30 min incubation at 37°C by adding 1.2 ml of 5% trichloroacetic acid. It was centrifuged at 13,000 g for 15 min. The supernatant was made up to 4 ml and read at 280 μm with a spectrophotometer. A 0.005% solution of tyrosine was used as standard for calculating μg of tyrosine liberated.

For determining amylase activity, the method of Noelting and Bernfeld (1948) was modified. A reaction mixture containing 0.2 ml enzyme extract, 0.2 ml Tris-HCl buffer (pH 8.2) and 0.4 ml 1% starch solution was incubated at 37°C for 20 min. The reaction was stopped by adding 1.2 ml 3,5-dinitrosalicylic acid reagent and heating at 100°C for 5 min. The absorbancy of the solution after diluting it to 4 ml was read at 550 μm and calculated μg of maltose equivalents liberated using 0.01% maltose solution as standard.

2.5 Time-course and dose-response studies

The time-course of digestive enzyme secretion by midgut epithelial extract was studied by incubating the midgut preparations in a medium containing extract of 2 midgut epithelia/10 ml solution for 10, 30 and 60 min. Digestive enzyme secretion with respect to homogenate concentration was studied by incubating the midgut preparations in incubation solutions of varying concentrations of midgut epithelia (1, 2, 4 and 6) for 30 min. In both cases, the contents of the midgut preparations were collected for quantitative estimation of protease and amylase.

2.6 Control experiments

Casein (50 mg) and starch (50 mg) were added to 10 ml insect saline, mixed well and filtered through glass wool. The supernatant after centrifugation at 10,000 g for 10 min was used to incubate midgut preparations in control experiments.

3. Results

The contents of midgut preparations incubated in a medium containing extract of 2 midgut epithelia/10 ml incubation solution for 30 min showed significant increase in protease (3.61 ± 0.81) and amylase (11.03 ± 1.44) levels when compared with controls (protease: 1.86 ± 0.85; amylase: 7.95 ± 1.74). When incubation period was increased from 10–60 min, increased secretion of protease and amylase was noticed (table 1). Secretion of protease and amylase also increased with increasing concentration of the midgut epithelial extract i.e, from 1–6 midgut epithelia/10 ml solution (table 2).

4. Discussion

In vertebrates, hormones secreted by the gastro-entero-pancreatic system are involved in several digestive processes. The existence of a comparable system in insects is supported by ultrastructural and immunological studies. Basal granulated cells with ultrastructural characteristics of endocrine cells of the vertebrate gastro-

Table 1. Time-course of digestive enzyme secretion by midgut epithelial extract of *O. rhinoceros*.

	Incubation time (min)					
	10		30		60	
	Control	Test	Control	Test	Control	Test
Protease activity[a]	1.33 ± 0.60 (8)	2.55 ± 1.50 (10)	1.86 ± 0.85 (10)	3.61 ± 0.81^c (10)	2.43 ± 0.81 (8)	5.77 ± 1.67^c (8)
Amylase activity[b]	7.16 ± 1.20 (8)	8.67 ± 2.23 (8)	7.95 ± 1.74 (10)	11.03 ± 1.44^c (10)	8.67 ± 1.54 (8)	15.48 ± 3.76^c (8)

[a] μg of tyrosine liberated.
[b] μg of maltose equivalents liberated.
[c] Significance at 0.001 level.
No. of determinations given in parentheses.

Table 2. Effect of midgut homogenate concentration on digestive enzyme secretion.

	Homogenate concentration (No. of midgut epithelia per 10 ml incubation solution)				
	Control	1	2	4	6
Protease activity[a]	1·86 ± 0·85 (10)	2·39 ± 0·63 (8)	3·61 ± 0·81[c] (10)	3·49 ± 0·79[c] (10)	3·71 ± 0·56[c] (6)
Amylase activity[b]	7·95 ± 1·74 (10)	9·86 ± 1·61 (8)	11·03 ± 1·44[c] (8)	12·12 ± 1·97[c] (8)	13·51 ± 2·25[c] (6)

Same notations as in table 1.

intestinal tract were identified in 7 species of Lepidoptera (Endo and Nishiitsutsuji-Uwo 1981). *P. americana* (Nishiitsutsuji-Uwo and Endo 1981), *Oryctes nasicornis* (Bayon 1981) and *Aedes aegypti* (Brown *et al* 1985). Similarly, the midgut epithelium of insects also revealed the presence of peptides immunologically related to those of the vertebrate gastrointestinal tissue such as insulin-like peptide in Hymenopteran insects (Ishay *et al* 1976), glucagon-like peptide in *Manduca sexta* (Tager and Kramer 1980) and pancreatic polypeptide-, somatostatin- and enteroglucagon-like immunoreactive materials in *P. americana* (Iwanaga *et al* 1981). In fact with an estimated 500 endocrine cells, the midgut of *A. aegypti* constituted the largest endocrine organ in an adult mosquito (Brown *et al* 1985). However, only very few reports are available on the probable functional role of the insect gut endocrine system.

In vitro studies revealed that in *Calliphora* 5-hydroxytryptamine stimulated fluid secretion by isolated salivary glands. The brain of this insect contained 5-hydroxy-tryptamine (Berridge and Patel 1968). Removal of salivary glands of 10 day old adult *P. americana* resulted in a gradual decline in midgut invertase secretion. No such effect occurred when salivary ducts were severed. Injection of salivary gland extract restored enzyme secretion to a large extent in salivarectomised insects, indicating the presence of a hormone like inducing factor (Agrawal and Bahadur 1981). In the adult *P. americana* the midgut extracts also contained a substance which increased protease in the midgut lumen 25–30 min after injection of the extract into the haemocoel, although significant values were obtained only around sun set (Rounds 1968). The results presented here revealed that in the third instar larvae of *O. rhinoceros* extracts of midgut epithelium were effective in increasing digestive enzymes in gut contents. The effect of the midgut epithelial extract on digestive enzyme secretion was both time and dose dependent. This observation suggests that in *O. rhinoceros* the midgut itself is functioning as an endocrine organ, regulating the secretion of digestive enzymes without the intervention of other endocrines or nervous components. The secretory process of a midgut endocrine cell may take place in response to chemical stimulation in the gut lumen or mechanical stimulation in the epithelium (Iwanaga *et al* 1981). Endocrine cells of the 'open type' were described in most of the insects studied (Nishiitsutsuji-Uwo and Endo 1981; Brown *et al* 1985). Such cells may be specialised for the reception of molecular signals from the food ingested by the animal (Brown *et al* 1985). In the endocrine cells of the midgut of *A. aegypti* phenylalanine-methionine-arginine-phenylalanine-amide (FMRFamide) and pancreatic polypeptide (PP) like immunoreactivity decreased within 6 h after ingestion of blood suggesting release of hormones (Brown *et al* 1986). The hormones (peptides of

monoamines) released into the haemolymph by gut endocrine cells are believed to be performing a regulatory role in many digestive and metabolic processes. While some of these hormones may have paracrine effects on regenerative cell differentiation, enzyme production by the digestive cell and working of the inner circular muscle of the midgut, others may have endocrine effects on the activity of more distant target tissues such as the central nervous system and fat body (Iwanaga *et al* 1981; Brown *et al* 1985). The enhanced activity of protease and amylase in the contents of midgut preparations incubated with midgut epithelial extract, observed in the present study, thus appears to be due to the action of a midgut hormone stimulating digestive enzyme release into the lumen of the gut. Both the source and the target organ of this hormone were found to be the midgut epithelium and for this reason, it may come under the group of paracrine hormones.

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club-shaped sensory hair on the first instar *Ectropis excursaria* epidoptera: Geometridae) and its role in the behaviour of the erpillars

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Abstract. A unique club-shaped hair on the first instars of an Australian geometrid caterpillar is described. The sensory hairs occur on the body segments and is absent on the head and the last abdominal segment. These hairs have different orientations and are replaced by filiform hairs after the first moult. Regression lines describing the movement of hairs of different orientations in relation to a force is presented. With these results the deflection in the sensory hair due to gravity when the caterpillar is on a vertical plane was calculated. These calculations suggest that the hairs may not function as gravity receptors. The possible role of these hairs in the behaviour of caterpillars is discussed.

Keywords. Club-shaped hair; first instar; morphology; distribution; orientation; spring constant; torque; deflection; gravity receptors.

Introduction

nays *et al* (1976) reported the occurrence of a unique club-shaped sensory hair on first instars of locusts *Schistocerca gregaria*. The authors also investigated the ervation of these hairs. However, behavioural significance of these hairs is not wn. Sensory hairs of similar characteristics were discovered on the first instar vae of an Australian geometrid caterpillar, *Ectropis excursaria*. The morphology, ribution and certain physical properties of the hair is described here. Its possible e in the behaviour of first instars is discussed.

Materials and methods

Morphology and distribution of the sensory hair

shly killed first instars coated with carbon under vacuum were examined under a nning electron microscope.

Measurement of the deflection of hair due to force

e force required for deflection of the sensory hair was determined in the following nner. A freshly killed first instar was mounted on an entomological pin, bent in 'L' pe, with paraffin wax. The caterpillar was mounted on its ventral side with its gitudinal axis parallel to the longitudinal axis of one of the bent sides. The insect s mounted was viewed under a binocular microscope with a graticule (7 mm = 100 isions). A single sensory hair on the lateral side was made to coincide with a

division on the graticule. Compressed air lead through a tube and the sensor head of a thermo-anemometer was placed close to the insect holder (figure 1A). The entire set-up was fixed to a table to reduce movement. Air was released slowly through a regulator and when the hair deflected such that its tip coincided with the next division on the graticule, the air-speed was recorded. If the length of the hair is known (measured with the same graticule) then the angle of deflection of the hair follows, amplitude of excursion of the hair tip under the influence of gravity/length of the hair = sine angle of deflection of the sensory hair (figure 1B). Deflections of 3 hairs were recorded from an insect to avoid errors due to desiccation. The deflection of the hairs of different orientations with the wind force from the anterior and posterior end of the insect was studied.

In another set of experiments, the maximum possible deflection of a sensory hair was studied by slowly releasing the air until no more deflection of the sensory hair occurred.

3. Results

3.1 *Morphology of the hair*

Each hair consists of a shaft which widens distally and ends in a slightly bulbous tip. The shaft has a surface layer and internal frames which gives the appearance of longitudinal grooves along the shaft. The base of the hair is attached to the body through a hole on the top of smooth dome. The diameter of the hole on the dome is much larger than the base of the hair permitting a large deflection of the hair (figure 2C). The bulbous tip appears to be formed by a membrane, filled with fluid, as evident from collapsed tip of the sensory hair on a freeze dried insect (figure 2E). The dimensions of the hair is presented in table 2.

(A)   **(B)**
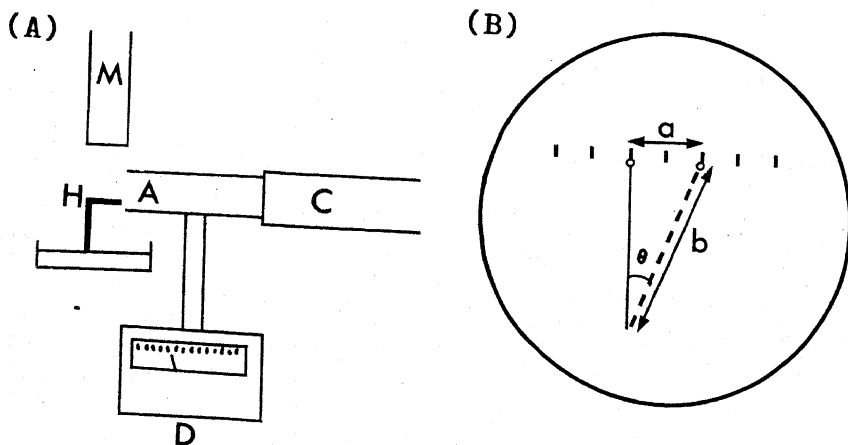
Figure 1. A. Experimental design for studying the relationship between force and deflection of the sensory hair (M, microscope; H, insect holder; C, inlet for air; A, hot wire anemometer; D, dial of the anemometer). **B.** Diagram of the view through the microscope (a, amplitude of excursion of the hair tip under the influence of gravity; b, length of the hair; θ, deflection of the hair).
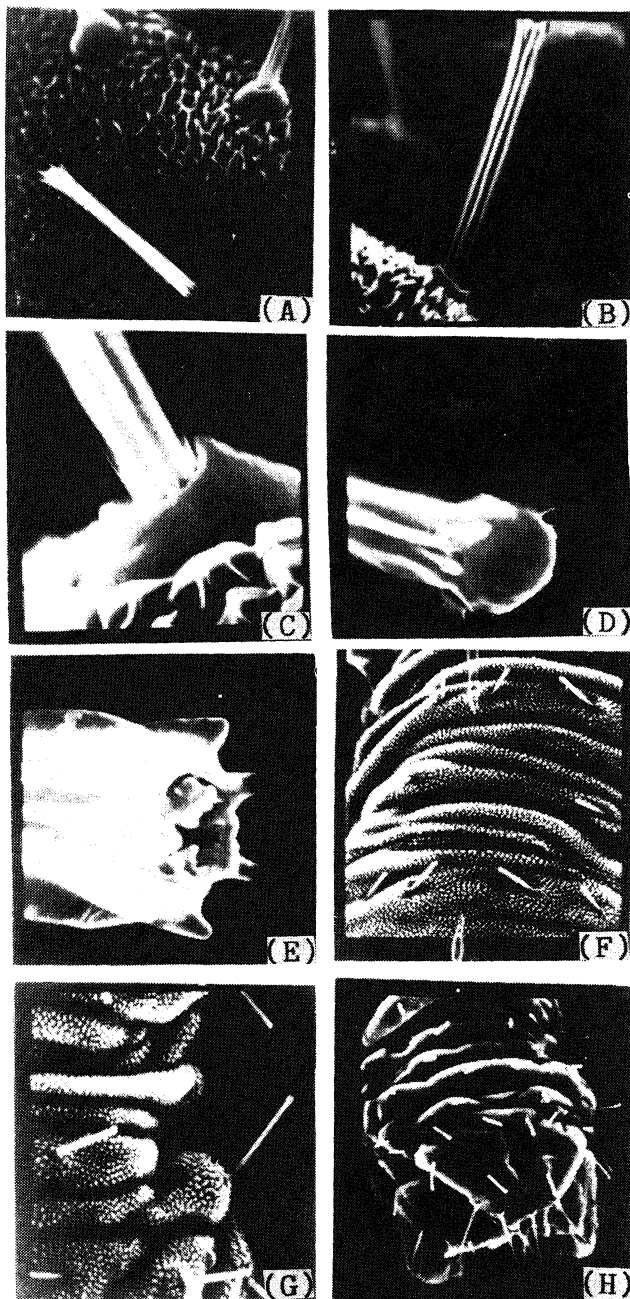
Figure 2. **A and B.** A club-shaped sensory hair (**A.** 10 mm = 14 μm. **B.** 10 mm = 7·6 μm).
C. Base of the hair (10 mm = 17 μm). **D.** Bulbous tip (10 mm = 15 μm). **E.** Tip of the hair on
a freeze dried insect (10 mm = 0·9 μm). **F.** Arrangement of the hair on the dorsal side of the
insect's body (10 mm = 30 μm). **G.** Hairs on the lateral side (10 mm = 44 μm). **H.** Filiform
hairs on the posterior end of the insect (10 mm = 53 μm).

3.2 *Distribution of the sensory hair*

The club-shaped hairs are distributed on all the thoracic and abdominal segments except the last segment which has filiform hairs (figure 2H). The head also bears only filiform hairs. The frequency of the hair with respect to their position on the body is presented in table 1.

The club-shaped sensory hairs occurred only in the first instars. After the first moult, caterpillars bore only filiform hairs, furthermore there was also a reduction in the number of hairs on the body.

3.3 *Orientation of the sensory hair*

The hairs on the lateral side of the insect had 3 characteristic orientations. (i) Hairs inclined towards the head: Such hairs are more frequent on the anterior end of the body. (ii) Hairs inclined towards the posterior end: These are more frequent on the posterior end. (iii) Hairs perpendicular to the surface of the insect's body: Such hairs are distributed all over the body. On the dorsal surface of the caterpillar's body, the hairs are arranged in alternate rows of 4 and 2 hairs and are inclined towards the mid-dorsal line (figure 2F).

3.4 *Geometrical parameters of the hair*

The geometrical parameters of the hairs were calculated assuming the stalk of the hair to be a cylinder and the bulbous tip to be a sphere. The volume and mass of the hair is given by the sum of the volume and mass of the stalk and sphere, respectively. Alternatively, these parameters were also calculated by assuming the stalk to be frustrum. The geometrical parameters of a typical hair on the lateral and dorsal surface is presented in table 2.

Table 1. Frequency of sensory hairs in the different parts of the caterpillar's body.

	Lateral	Dorsal	Ventral
Anterior	19±4	8±1	0
Middle	24±3	17±2	16±4
Posterior	17±1	9±2	0

$n = 10$.

Table 2. Geometrical parameters of the hair.

Parameters	Dorsal/ventral	Lateral	n
Length of the hair (μ)	21±2·0	29±3·0	20
Diameter of the hair (μ)	2±0·3	3±0·9	20
Volume of a typical hair (μ^3)	320·22	1584·63	—
Mass of a typical hair (mg)	$3·0 \times 10^{-7}$	$1·7 \times 10^{-7}$	—

Density of the hair = mean of the density of cuticle ($1·2 \times 10^3$ kg/m^3, Jenson and Weis-Fogh 1962) and density of haemolymph ($1·05 \times 10^3$ kg/m^3, Altmann and Dittmer 1975). The volume of chitin and haemolymph in the sensory hair has been considered to be equal.

3.5 *Deflection of the sensory hair in relation to force*

The deflection of the sensory hair depended on the magnitude of the force applied, the relationship being linear (linear regression significant, figure 3). However, the slopes of the regression lines for hairs of different orientations with different directions of airflow was significantly different (Analysis of covariance, $F = 23.77$, $P < 0.05$) suggesting a difference in sensitivity and a preferred direction of movement in the hairs with different orientations. Thus the lateral straight hairs with airflow
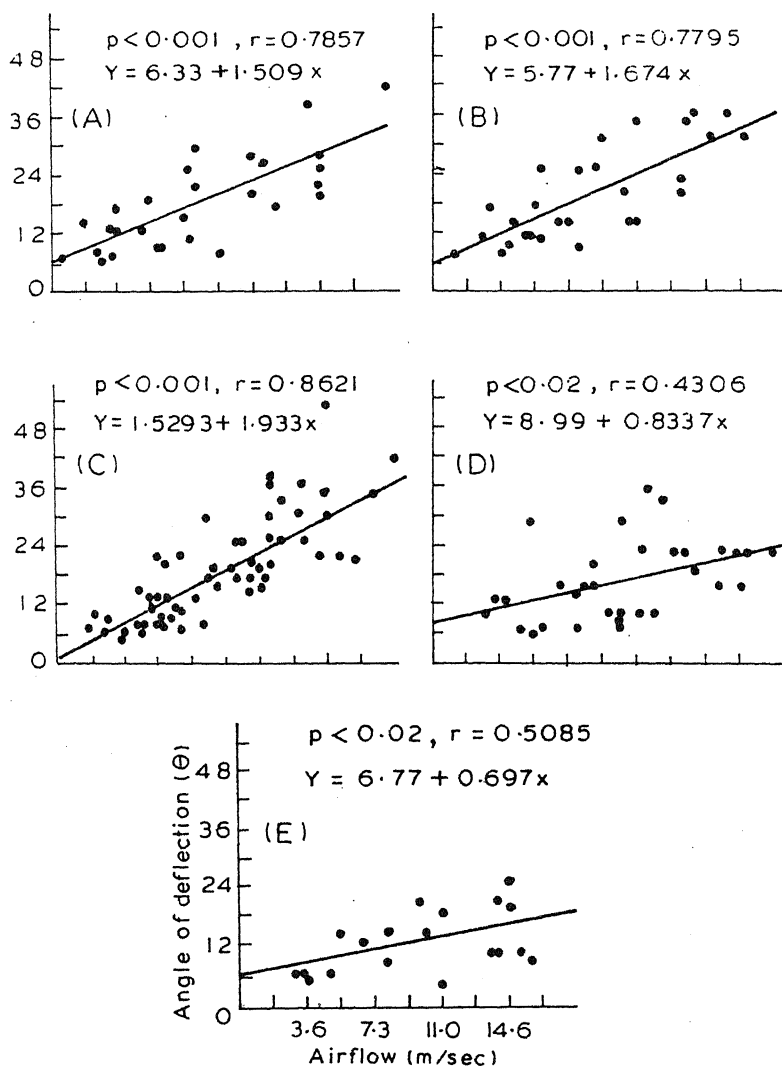
Figure 3. Relationship between the angle of deflection (degrees) of the sensory hair and air flow (m/s). **A.** Lateral perpendicular hairs with air flow from anterior end of the insect. **B.** Lateral perpendicular hairs with air flow from posterior end. **C.** Hairs inclined forward with air flow from anterior end of the insect. **D.** Hairs inclined towards the posterior end with air flow from posterior end. **E.** Any direction with air flow from opposite side of the hair's inclination.

from anterior and posterior end of the insect and hairs inclined anteriorly with
airflow from the anterior end of the insect had similar slopes indicating a similarity in
their sensitivity to force. However, hairs inclined posteriorly with airflow from the
posterior end of the insect and hairs inclined away from the direction of airflow had
slopes smaller than the other hairs ($P < 0.001$) suggesting less sensitivity to force.

Maximum deflection possible in a sensory hair also depended on orientation of the
hair. Hairs perpendicular to the body of the insect deflected 26° and 28° when airflow
was provided from the anterior and posterior end, respectively (table 3). Inclined
hairs showed a greater deflection when the airflow came from the side towards which
the hairs were inclined than when the airflow was from the opposite end (table 3).

Based on the movement characteristics of the sensory hair i.e. a linear increase in
deflection as the force was increased and a threshold degree beyond which no further
deflection of the hair occurred, the root of the sensory hair can be assumed to be of
the type given in figure 4. The hair is assumed to have a fixed axis on which it
deflects. For a structure of the above type the amount of deflection that would occur
due to any force follows the equation, torque/deflection = spring constant. Spring
constant was calculated (appendix) for a hypothetical hair of 20 and 90 μm length
and 1 and 8 μm diameter. Torque produced by the acceleration due to gravity
(9.8 m s^{-2}) was computed using different values of spring constant and the possible
deflection produced by gravity was calculated (table 4, see appendix for steps in
calculation).

Table 3. Maximum possible deflection in the sensory hair of different orientations.

Orientation of the hair	Direction of the airflow	Deflection (degree)
Perpendicular to the body	Anterior end of the insect	$26 \pm 9a$
Perpendicular to the body	Posterior end of the insect	$28 \pm 9a$
Inclined towards the head	Anterior end	$28 \pm 11a$
Inclined towards the posterior end	Posterior end	$27 \pm 6a$
Any direction	From opposite direction of the inclination	$15 \pm 6b$

Means not followed by the same letters are statistically significant (one-way ANOVA, $P < 0.05$, means
compared by Tukey test).

(A)   **(B)**
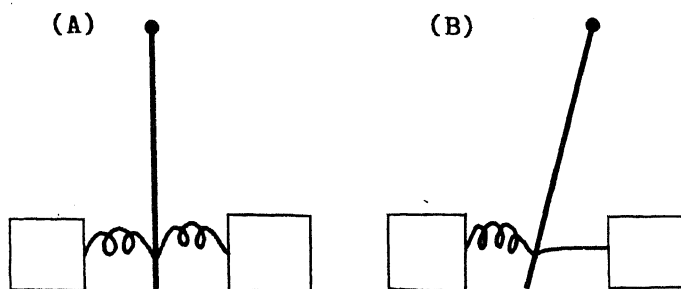
Figure 4. Hypothetical hair root model. **A.** Normal position. **B.** When force is applied

Table 4. Effect of gravity on the deflection of sensory hair.

Hair dimensions (μ)			Deflection
Length	Diameter	Spring constant	(degree)
20	1	$1{\cdot}8 \times 10^{-10}$	$2{\cdot}0 \times 10^{-5}$
20	8	$9{\cdot}1 \times 10^{-12}$	$6{\cdot}9 \times 10^{-4}$
90	1	$3{\cdot}0 \times 10^{-10}$	0·04112
90	8	$1{\cdot}5 \times 10^{-10}$	0·0825

4. Discussion

One of the characteristic behaviours of the newly emerged first instar *E. excursaria* is upward climbing which has been shown to be in response to light and gravity (Ramachandran 1987b). The mechanism of larval phototaxis is well investigated but that of negative geotaxis is unknown. Sandeman (1976) suggested that loaded hairs such as the ones described on *E. excursaria* and *S. gregaria* (Bernays *et al* 1976), could bend under the influence of gravity when the organism is on a vertical plane. Furthermore, this bending would increase when the insect is in motion upwards due to the inertial forces of the heavier tip. This produces a directionality in the deflection of the sensory hair with which it is possible to measure changes in the position of the animal from one point in its environment to another, with respect to gravity. This hypothesis was tested in the present study by modelling the movement of the sensory hair on *E. excursaria* by gravity. The maximum possible deflection due to gravity of the sensory hair, as calculated by considering the spring constant of the sensory hair, was only 0·0825° for a hair dimension of $90 \times 8\ \mu m$, these dimensions being far greater than the actual dimensions of the sensory hair ($50 \times 4\ \mu m$). The degree of deflection is less than the threshold deflection of 0·1° required to produce any electrophysiological response in a lepidopteran sensory hair (Tautz 1977). Thus, it appears that these hairs may not have a role in gravity perception. This conclusion is further supported by the finding that all the larval stages of *E. excursaria* except the pre-pupal stage respond to gravity (Ramachandran 1987b), however, only the first instars possess these club-shaped hairs.

Sensory hairs with identical morphological features have been reported to occur on the first instar *S. gregaria* (Bernays *et al* 1976). The difference in the characteristic of hairs on these two insects appears to be in their distribution and orientation. In locusts, these hairs occur only on the antero-dorsal surface and the head. It has been hypothesised that in *S. gregaria* since the vermiform larvae shed their embryonic cuticle after it has dug out of the soil, these hairs monitor the stimulus (a lack of all-round contact) to ecdysis (Reynolds 1980). In *E. excursaria*, hatching (escape from the egg shell) and eclosion (escape from the embryonic cuticle) occurs at the same time, precluding a function similar to the one suggested for locusts.

The first instars of *E. excursaria* disperse by wind (Ramachandran 1987a). Prior to the dispersal, insects assume a characteristic stance in which only the claspers are attached to the substrate and the rest of the body is held free. In the presence of wind force, the insect releases its hold on the substrate and is launched. Such a means of dispersal requires monitoring of wind velocity, for it has been observed that only wind forces of certain velocities dislodge the insect. Such a wind dispersal does not

occur in the other instars. Thus it appears that these sensory hairs may be involved in anemoreception.

Acknowledgements

Appendix

Steps in the calculation of deflection in the hair due to gravity.

1. Calculate the Reynolds number for the sensory hair, Reynolds number $= ud/v$, u is force at any point on the hair, d is diameter of the hair and v is kinematic viscosity of the air ($1 \cdot 5 \times 10^{-5}$ m).

2. If the Reynolds number is $\leqslant 30$, then the drag coefficient of the sensory hair follows, K/Re, $K =$ constant.

3. Force on the hair is given by equation, $F = dc\,(l \times d)\,(1/2 \times p \times V^2)$, l is length of the hair, dc is drag coefficient, P is air density ($1 \cdot 204$ kg/m^3) and V is velocity of the wind force used [velocity required to produce unit deflection in the sensory hair calculated from the regression equation for straight hairs with wind force from the anterior end has been used (figure 3A)].

4. Torque $=$ force $\times$ (length of the hair/2).

5. Torque/deflection $=$ spring constant of the hair.

6. Torque for acceleration due to gravity $=$ mass of the hair $\times$ acceleration due to gravity.

From step 5 spring constant of the hair in the experimental condition is calculated. For degree of deflection due to gravity, deflection $=$ torque due to gravity (step 6)/spring constant (step 5) (Sabersky *et al* 1971).

Assumptions and limitations of the calculation

The torque on the hair root has been calculated using the linear relationship between air velocity and deflection of the sensory hair. But the air velocity measured here is only an approximation as the measurement was not made near the hair itself.

Torque on the hair has been calculated by assuming the hair to be a cylinder with its equilibrium point half way long its length.

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Experimental study on addition of superphosphate and mahua oil cake for increased yield from fish ponds

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Abstract. Survival, growth and reproduction of *Cyprinus carpio* were studied in ponds treated with mahua oil cake and single superphosphate. The results indicated that single superphosphate (500 kg/ha) compensated for the deleterious effects of mahua oil cake (2,000 kg/ha) on survival, growth and reproduction of fish. Better fecundity-weight relationship of fish and bottom organisms were evident at the same input levels. In order to maintain ammonia concentration in ponds below the toxic threshold, application of single superphosphate seems to be essential. For better fish culture management, use of mahua oil cake in high amount or its frequent addition should be avoided.

Keywords. Mahua oil cake; single superphosphate; survival; growth; reproduction; *Cyprinus carpio*; fish ponds.

1. Introduction

Application of mahua oil cake (MOC) in fish culture in India play an important role for removing predatory fishes, unwanted aquatic life and also for increasing the fertility of soil and water significantly. The efficiency of this cake has been evaluated for removing aquatic fauna and better management of fish ponds (Bhatia 1970; Jhingran 1983), toxicity, survival, growth and reproduction of fish (Lakshman 1983; Sarkar 1984, 1985; Sarkar and Pramanik 1987) and increasing soil fertility since it is considered as a source of nitrogen (Anonymous 1959; Misra and Sen 1959; Iswaran and Sen 1960). Recently, the importance of this oil cake in nursery and rearing ponds has been considered precisely (Sarkar 1985). Indiscriminate use of MOC in fish ponds not only involves its wastage, resulting in organic pollution (Sarkar 1987) but also costs more for fish culturists (Sarkar 1985; Sarkar and Pramanik 1987). Although different types of phosphatic fertilizers are generally used for intensive fish cultivation in ponds (Dobbins and Boyd 1976; Lichtkoppler and Boyd 1977; Boyd 1979; Boyd *et al* 1981) only single superphosphate (SSP) is extensively used by Indian farmers in fish culture alongwith oil cakes and nitrogenous fertilizers (Ghosh *et al* 1979; Sarkar 1983) since phosphorus fertilizers increase the concentration of available phosphorus in soil and water, thereby phytoplankton production also increased significantly (Boyd *et al* 1981). Sarkar and Pramanik (1987) demonstrated that lime exhibited an influence on the effects of MOC in fish reproduction. The present study deals with the influence of SSP on the effects of MOC on survival, growth and reproduction of fish in ponds.

2. Materials and methods

2.1 *Materials*

Commercial product of MOC and SSP were used in this study. Fingerlings of

Cyprinus carpio (103 ± 4.7 g) collected locally were used. Tests were run for 6 months in 10 ponds (water temperature ranged from 27·5–34·5°C) located at Sonamukhi of the District Bankura, West Bengal. Treatment of ponds (except control) with MOC and SSP were made in 5 equal instalments at 20 days interval. They were powdered prior to application. Rates of inputs and their combinations applied are shown in table 1. Prior to stocking of ponds with fish, ponds were netted out several times very carefully with the help of drag net in order to remove unwanted aquatic life as far as possible. Each pond was stocked with fish at a stocking density of 6,000 per hectare. Fishes were fed daily at the rate of 6% of body weight of the fish with fish meal marketted by Fair Agro Industries, Barrackpore, West Bengal.

2.2 Methods

At the end of experiment, fishes were collected by dewatering of ponds and the results were subjected to statistical analyses (Lagler 1959; Campbell 1967). The number of fish examined were 2782 and 2846, male and female respectively. Surface water samples were collected between 11 am and 2 pm at interval of 15 days (APHA 1975).

3. Results

The survival, growth and yield, condition factor (K) and gastro-somatic index (GSI) of fish are shown in table 2.

3.1 Survival

The survival rate of fish at different treatments varied significantly. Fish survival at PM1, PM2 and PM3 significantly increased by 25–41% ($P < 0.05$–0·01), at M4 it decreased by 18% and increased by 13–16% at M1, M2 and M3 ($P < 0.05$).

3.2 Growth and yield

Growth rate of fish varied significantly in different treatments. The weight increment

Table 1. Layout of the experiment.

Pond area (ha)	Mean ($\pm$ SE) depth (m)	Rate denoted	Treatment rate (kg/ha/yr)	
			P	M
0·02	$1·2 \pm 0·2$	C	—	—
0·03	$1·0 \pm 0·3$	P	500	—
0·01	$1·3 \pm 0·3$	M1	—	1,500
0·04	$1·2 \pm 0·5$	M2	—	2,000
0·02	$1·0 \pm 0·3$	M3	—	2,500
0·03	$1·4 \pm 0·01$	M4	—	3,000
0·02	$1·3 \pm 0·01$	PM1	500	1,500
0·02	$1·5 \pm 0·02$	PM2	500	2,000
0·01	$1·3 \pm 0·05$	PM3	500	2,500
0·03	$1·2 \pm 0·02$	PM4	500	3,000

C. Control; P, single superphosphate; M, mahua oil cake.

Table 2. Influence of MOC and SSP on the survival (%, A), yield ($\times$ 10 kg/ha, B), K and GSI of fish (mean $\pm$ SE).

Treatment	A	B	K	GSI
C	56 ± 1·05	134 ± 2·07	2·06 ± 0·054	8·90 ± 0·232
P	61 ± 1·02	149 ± 1·08	2·21 ± 0·059	8·88 ± 0·231
M1	65 ± 1·07	168 ± 2·08	2·01 ± 0·052	8·80 ± 0·228
M2	63 ± 1·00	166 ± 2·04	2·17 ± 0·057	8·86 ± 0·230
M3	63 ± 1·07	173 ± 1·08	2·05 ± 0·054	8·90 ± 0·236
M4	46 ± 1·08	142 ± 2·55	2·03 ± 0·052	8·92 ± 0·238
PM1	73 ± 1·05	183 ± 2·07	2·14 ± 0·060	8·87 ± 0·231
PM2	79 ± 1·08	198 ± 2·35	2·20 ± 0·058	8·81 ± 0·228
PM3	70 ± 1·03	195 ± 2·06	2·19 ± 0·56	8·81 ± 0·230
PM4	60 ± 1·06	193 ± 2·04	1·99 ± 0·050	8·94 ± 0·240

of individual fish was maximum in ponds at P, M1, M2, PM1 and PM2 (31–43%) and minimum at other treatments (8–15%). At P, PM1, PM2, M1 and M2, the growth was reduced at the early stages of fish followed by a rapid growth rate. The average weight of individual fish increased by 18–49% of control ($P < 0.05$) at different treatment rates.

The yield of fish at P and M4 increased by 6 and 11% respectively ($P > 0.05$). At other treatment rate the yield was significantly increased by 24–48% of control ($P < 0.05$–0.01). The yield was highest at PM2.

3.3 Condition factor and GSI

The condition factor (K) and GSI of fish at different treatments are shown in table 2. The K of fish at P, M2, PM2 and PM3 was significantly increased by 5–7% of control ($P < 0.05$). The GSI of fish at different combinations did not differ significantly from control ($P > 0.05$).

3.4 Length-weight regressions

Slopes of regressions of length and weight for fish at different treatment combinations varied significantly from control ($P < 0.05$–0.01, table 3).

3.5 Reproduction

The maturity index (MI) and fecundity of fish is shown in table 4. The MI of female fish significantly increased by 4–6% of control at P, M2, PM2, M3 and PM3. The MI of male fish did not exhibit any significant difference in any treatment from control ($P > 0.05$). The mean values of fecundity of fish exhibited significant difference than control ($P < 0.05$). Ponds treated with PM2 exhibited greater increase (14% of control) in fecundity rate of fish. At M3, M4, PM3 and PM4, the fecundity rates significantly decreased by 13–18% than in control ($P < 0.05$).

Correlation coefficients between fecundity-length (FL), fecundity-weight (FW) and length-weight (LW) indicated significant correlation (table 5) between FL and FW than LW relationship. Table 5 shows that the FW relationship was better at C, M1,

Table 3. Influence of MOC and SSP on the LW regressions of fish ($Y = a + bX$).

Treatment	Cyprinus carpio
C	$-3.802 + 2.432$ X
P	$-5.247 + 3.266$ X
M1	$-5.046 + 3.192$ X
M2	$-4.359 + 2.818$ X
M3	$-3.427 + 2.028$ X
M4	$-4.710 + 2.992$ X
PM1	$-5.143 + 3.231$ X.
PM2	$-5.006 + 3.147$ X
PM3	$-6.027 + 3.798$ X
PM4	$-5.078 + 3.178$ X

Table 4. Influence of MOC and SSP on the fecundity (F) and maturity index (MI) of fish (mean $\pm$ SE).

Treatment	F	MI Male	Female
C	$15,743 \pm 36$	0.67 ± 0.021	2.11 ± 0.203
P	$16,075 \pm 43$	0.62 ± 0.020	2.19 ± 0.210
M1	$15,782 \pm 38$	0.69 ± 0.024	2.13 ± 0.204
M2	$15,693 \pm 40$	0.66 ± 0.020	2.21 ± 0.212
M3	$13,741 \pm 35$	0.60 ± 0.019	2.20 ± 0.210
M4	$13,185 \pm 38$	0.63 ± 0.024	2.12 ± 0.215
PM1	$15,682 \pm 42$	0.67 ± 0.023	2.10 ± 0.207
PM2	$17,872 \pm 50$	0.70 ± 0.024	2.23 ± 0.215
PM3	$12,887 \pm 45$	0.71 ± 0.025	2.19 ± 0.212
PM4	$13,024 \pm 43$	0.72 ± 0.027	2.10 ± 0.208

Table 5. Comparison of FL, FW and LW correlations of *C. carpio* in ponds.

Treatment (Item, r)	FL	FW	LW
C	0.756	0.744	0.707
P	0.714	0.887	0.692
M1	0.792	0.705	0.721
M2	0.721	0.896	0.666
M3	0.789	0.722	0.679
M4	0.748	0.746	0.598
PM1	0.701	0.905	0.603
PM2	0.695	0.927	0.611
PM3	0.821	0.708	0.579
PM4	0.807	0.717	0.590

M3, M4, PM3 and PM4 ($P < 0.05$) but at other treatment combinations, the FW relationship should be preferred. The relationship between FW is highest at PM2 ($P < 0.01$).

3.6 Aquatic ecosystem

The effects of different treatment combinations of MOC and SSP on water parameters, plankton community, aquatic weeds and bottom organisms are shown in tables 6 and 7.

3.6a *Water parameters*: Table 6 indicates that the temperature of water at different treatment rates increased by 3–7% of control ($P > 0.05$) while pH increased by 18–31% of control ($P < 0.05$). Free carbon dioxide significantly decreased by 41–71% of control ($P < 0.01$). Dissolved oxygen significantly increased by 28–70% of control ($P < 0.05$–0.01) at different treatment rates. Total alkalinity of water at M3, M4, PM3 and PM4 significantly decreased by 15–20% of control and increased by 4–20% of control ($P < 0.05$). Ammonia-nitrogen concentration of water at M3 and M4 significantly increased by 161–194% of control ($P < 0.01$) and decreased by 22–44% of control ($P < 0.05$) at PM1, PM2, PM3 and PM4.

3.6b *Plankton community*: Different species of phytoplankton, zooplankton and their abundance (collected by filtering 30 l of water through plankton net No. 21)

Table 6. Influence of MOC and SSP on various parameters of pond water (mean ± SE).

Treatment	pH	Free CO_2 (ppm)	Temperature (°C)	Dissolved oxygen (ppm)	Total alkalinity (ppm)	Ammonia nitrogen (ppm)
C	6·7 ± 0·08	3·4 ± 0·07	30·8 ± 0·2	6·9 ± 0·05	286 ± 4	0·18 ± 0·03
P	8·2 ± 0·07	1·8 ± 0·05	31·7 ± 0·4	8·9 ± 0·04	298 ± 3	0·19 ± 0·05
M1	8·5 ± 0·06	1·9 ± 0·04	32·5 ± 0·6	9·7 ± 0·06	315 ± 5	0·17 ± 0·05
M2	8·5 ± 0·04	2·0 ± 0·03	33·0 ± 0·2	10·5 ± 0·03	319 ± 5	0·17 ± 0·04
M3	8·7 ± 0·05	1·5 ± 0·05	31·7 ± 0·4	8·8 ± 0·05	242 ± 5	0·47 ± 0·05
M4	8·0 ± 0·03	1·4 ± 0·03	32·7 ± 0·6	9·6 ± 0·02	233 ± 6	0·53 ± 0·04
PM1	7·9 ± 0·04	1·8 ± 0·04	33·0 ± 0·3	11·2 ± 0·04	342 ± 6	0·14 ± 0·04
PM2	8·6 ± 0·05	1·5 ± 0·02	32·7 ± 0·6	10·5 ± 0·04	327 ± 5	0·12 ± 0·04
PM3	8·5 ± 0·05	1·0 ± 0·04	31·9 ± 0·4	11·7 ± 0·03	228 ± 5	0·14 ± 0·03
PM4	8·8 ± 0·04	1·3 ± 0·04	32·1 ± 0·4	10·8 ± 0·02	243 ± 4	0·10 ± 0·05

Table 7. Influence of MOC and SSP on the abundance of plankton, bottom organisms (g/m^2) and aquatic weeds (kg/m^2) in ponds (mean ± SE).

	Plankton		Bottom organisms			Aquatic weeds	
Treatment	Phytoplankton	Zooplankton	Oligochaetes	Insect larva	Gastropods	*A. pinnata*	*L. minor*
C	158 ± 4·05	218 ± 3·06	0·7 ± 0·03	11·5 ± 0·8	4·5 ± 0·02	1·5 ± 0·02	0·9 ± 0·1
P	217 ± 6·11	224 ± 4·03	1·2 ± 0·07	10·9 ± 0·5	4·4 ± 0·04	—	—
M1	241 ± 5·08	229 ± 3·12	0·4 ± 0·02	11·3 ± 0·3	4·5 ± 0·03	—	—
M2	198 ± 3·14	246 ± 4·13	1·3 ± 0·04	11·6 ± 0·6	4·5 ± 0·02	—	—
M3	217 ± 4·17	269 ± 3·11	1·4 ± 0·02	8·3 ± 0·4	5·8 ± 0·04	3·6 ± 0·06	1·7 ± 0·3
M4	230 ± 3·22	293 ± 4·11	0·2 ± 0·02	7·9 ± 0·2	6·3 ± 0·06	2·4 ± 0·08	1·5 ± 0·5
PM1	230 ± 4·11	234 ± 5·08	1·3 ± 0·03	12·0 ± 0·6	4·3 ± 0·03	—	—
PM2	288 ± 4·14	317 ± 3·07	1·6 ± 0·06	11·5 ± 0·7	4·6 ± 0·06	—	—
PM3	204 ± 3·15	288 ± 4·12	0·2 ± 0·02	8·5 ± 0·5	6·3 ± 0·03	3·0 ± 0·05	1·9 ± 0·5
PM4	243 ± 4·16	278 ± 2·77	0·2 ± 0·01	7·2 ± 0·6	5·8 ± 0·04	2·1 ± 0·03	2·0 ± 0·6

indicated that the phytoplankton concentration significantly increased by 25–82% of control ($P < 0.05$–0.01) while zooplankton concentration increased by 13–45% of control ($P < 0.05$–0.01) at M2, M3, **M4**, PM2, PM3 and PM4 and marginally increased by 3–7% of control ($P > 0.05$). The dominant forms of phytoplankton include *Navicula* spp. (20%), *Anabaena* sp. (15%), *Oscillatoria* spp. (28%) and *Phaecus* spp. (37%) and zooplankton *Brachionus* spp. (15·3%), *Cyclops viridis* (10·8%), *Daphnia magna* (17·2%), *Moina micrura* (15·3%), *Diaptomus* spp. (11·4%) and *Nauplii* (30%).

3.6c *Aquatic weeds*: Except at PM1, PM2, M1, M2 and P, different species of aquatic weeds (particularly *Lemna minor* and *Azolla pinnata*) grew in ponds at other treatment rates. At M3, M4, PM3 and PM4, *L. minor* significantly increased by 40–140% and that of *A. pinnata* by 67–122% of control ($P < 0.05$–0.01, table 7).

3.6d *Bottom organisms*: Table 7 indicates that oligochaete (*Brachiura sowerbyi*) significantly increased by 71–129% at P, M2, M3, PM1 and PM2 ($P < 0.01$) and decreased by 43–71% of control at other treatment rates ($P < 0.05$). Gastropods (*Lymnae leuteola, Planorbis exustrus*) also increased by 31–40% of control at M3, M4, PM3 and PM4 ($P < 0.05$), at these treatment rates insect larvae (*Chironomus* spp., *Chaoborus* sp. and dragon fly nymph) significantly decreased by 26–37% of control ($P < 0.05$). The overall concentration of bottom organisms were highest at M2 and PM2 only.

4. Discussion

Survival of fish increased at 1,500–2,500 kg/ha MOC but addition of single superphosphate at the rate of 500 kg/ha further enhanced fish survival. Obviously, SSP addition reduced the effects of MOC on fish survival. Application of MOC and SSP also enhanced the condition factor of fish. Therefore, in order to improve fish production in ponds, addition of oil cake plus phosphatic fertilizer seems to be advisable since fish production data reveals that the yield of fish was maximum in ponds treated with MOC and SSP at 2,000 and 500 kg/ha respectively.

The fecundity and MI of fish was higher in ponds where 2,000 kg/ha of MOC was added, but the addition of SSP (500 kg/ha) resulted in further improvement. Sarkar and Pramanik (1987) observed that the reproductive capacity of fish increased by the application of lime (1,125 kg/ha) alongwith MOC (2,000 kg/ha). Lakshman (1983) also observed significant increase in the fecundity rate of fish in ponds exposed to high rate of MOC with or without lime. Any decrease in the fecundity and MI of fish is due to the accumulation of toxic products like ammonia and saponin (mowrin) in water since MOC contains about 6% saponin, 2·6% of N, 0·8% of P_2O_5 and 1·8% of K_2O. Obviously, the application of MOC at the rate of 2,500–3,000 kg/ha supplies 62·5–75·0 kg/ha N to pond water. The toxic effects of MOC is due to liberation of ammonia and saponin in water. Since ammonia exhibits a wide variety of biological influence on fish life (Cairns *et al* 1975; Burkhalter and Kaya 1977; Sarkar and Konar 1985b; Sarkar and Pramanik 1986) it is reasonable to predict that excess of ammonia results in the reduction of the reproductive potential of fish in ponds, as high levels of ammonia are known to decrease reproduction in fish (Sarkar and Konar 1985a, b).

Correlation coefficient studies reveal that the FW relationship in ponds treated

with SSP (500 kg/ha) and MOC (2,000 kg/ha) significantly increased.

On addition of high rates of MOC, aquatic weeds and biomass production decreased but addition of SSP increased their production, the latter thus exhibiting synergistic impact on growth of aquatic weeds significantly.

Dissolved oxygen and ammonia concentrations of pond water after addition of different rates of MOC increased. Although the fluctuation of dissolved oxygen may not be directly responsible for any adverse impact on the reproduction of fish, the effect of ammonia concentration is important. Increase in ammonia concentration was followed by drastic reduction in the reproductive potential of different species of fish (Sarkar and Pramanik 1987; Sarkar 1987). It has been observed that a decrease in dissolved oxygen concentration enhanced ammonia toxicity (Merkens and Downing 1957; Thurston *et al* 1981). In ponds treated with MOC, initially there was biodegradation of the cake, resulting in increase in ammonia level. The addition of SSP lowered the ammonia level, exhibiting antagonistic influence on ammonia toxicity. The application of SSP in fish culture thus appears to be essential for maintaining water quality as well as reproductive potential of fish. Since chronic ammonia poisoning is a great problem in fish culture schemes (Spotte 1970), resulting in damage gills, liver, intestine and kidneys (Flis 1968) in addition to causing various diseases (Burrows 1964; Smart 1976) it is reasonable to assume that frequent addition of different oil cakes at high rates will result in overproduction of ammonia and these should either be avoided or additional phosphatic fertilizers should be made available to compensate the deleterious effects of oil cake.

Acknowledgements

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Free phenol and the improved tolerance in four Meloidogyne (root-knot nematode) susceptible hosts

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Abstract. One hundred ppm of phenol in 2% NPK fertilizer treatments of 4 *Meloidogyne incognita* susceptible hosts, reduced the pathogenic impact and improved their tolerance.

Keywords. Host tolerance; susceptibility ratings; environmental resistance factor; metabolic compatibility.

1. Introduction

Plant metabolism and the complex wood chemistry imply the role of phenols in the plant's structure and function (Cowling and Horsfall 1980). The role of phenols in plant pathogenesis and disease resistance are well documented (Acedo and Rhode 1971; Cowling and Horsfall 1980; Gibel 1974, 1982; Goodman *et al* 1967). Feldman and Hanks (1968, 1971) opened up the exciting field of improving the plant tolerances with phenolics. Soil amendments (Singh and Seetha Ramiah 1967, 1971) indicated the possible nematicidal roles of phenols through organic amendments.

Free phenol when introduced into the plant (Ribereau-Gayon 1972) is actively absorbed and metabolized due to the highly reactive −OH group in its ring. Phenols condense with the plant's metabolites (glycosides, uronides, proteins etc.), thereby alter the substrate relations of the nematodes (Feldman and Hanks 1971; McIntyre 1980), creating a physical barrier for the nematodes, making the roots unsuitable for the nematodes. They also inhibit the free enterprise of the nematode's enzymes (Wallace 1973) leading to starvation of nematodes, with consequent lesser infectivity of the pathogen (Van Gundy *et al* 1967).

The above features reveal the metabolic compatibility of phenol with regard to the host's metabolites and the pathogen's enzymes, involving its bidirectional roles on the host as well as the pathogen's enzymes.

In pilot experiments it was found that free phenol in NPK solutions inhibited the egg hatchings appreciably. Taking advantage of the above facts, 100 ppm of phenol in 2% NPK fertilizer solution was employed to assess the effect on 4 Meloidogyne (root-knot nematode) viable hosts.

2. Methodology

Seven day old seedlings (from surface sterilized seeds) of *Hibiscus canabinus*, *Phaseolus mungo*, *Hibiscus esculentus* and *Solanum melongena* maintained in 10 cm dia. plastic pots (1 kg sterile sandy loam-2 : 1) were inoculated with 10^3 *Meloidogyne incognita* juveniles. For each host, 12 pots were treated with 2% NPK solution and another set of 12, treated with 100 ppm phenol in 2% NPK solution. Moisture was

kept at 60% levels with the irrigants applied, thrice weekly. Forty five days after the inoculation, the experiments were closed and the plants assayed for the pathogen population (table 1) and redox enzyme activities (table 2).

The environmental resistance factor (ERF) as a measure of the resistance encountered by the pathogen was assessed as follows:

$$ERF = RR/RPi$$

When   RR = Rate of pathogen's reproduction,
          RPi = Rate of the population increase of pathogen.

$$RR = \frac{(P_f \div g \text{ root wt}) \div ♀ \text{ per g root}}{1 \times 100}$$

Table 1. Population dynamics of *M. incognita* in 4 susceptible hosts under the influence of phenol.

	H. canabinus		P. mungo		H. esculentus		S. melongena	
	UT	PT	UT	PT	UT	PT	UT	PT
Egg mass	35	24	29	21	23	16	20	15
Eggs/egg mass	530·2	382·6	435·4	370·2	325·6	240·8	275·8	188·3
Total eggs (10³)	18·56	9·18	12·63	7·77	7·49	3·85	5·52	2·83
Population (10³):								
Root	0·53	0·42	0·47	0·36	0·33	0·26	0·22	0·15
Soil	6·41	4·65	4·27	3·30	3·12	2·20	2·12	1·48
Total	25·50	14·25	17·37	11·43	10·94	6·31	7·86	4·46
RR	5·45	3·79	4·38	3·72	3·26	2·41	2·76	1·88
RPI	0·54	0·29	0·36	0·23	0·22	0·12	0·15	0·08
ERF total	10·09	13·07	12·17	16·17	14·82	20·08	18·4	23·5
ERF plant (P)	7·55	8·81	9·18	11·51	10·59	13·09	13·43	15·71
ERF soil (S)	2·54	4·26	2·99	4·66	4·23	6·99	4·97	7·79
ERF P/S	2·97	2·07	3·07	2·47	2·50	1·87	2·70	2·02

Values are average of 6 replicates (±0·01 to ±0·002).
UT, No phenol treatment; PT, phenol treated.

Table 2. Redox metabolism in 4 *M. incognita* susceptible hosts expressed as µg of TTC reduced per mg wt of tissues.

		H. canabinus		P. mungo		H. esculentus		S. melongena	
Enzyme activities		UT	PT	UT	PT	UT	PT	UT	PT
TDH	Root	375·8	310·4	298·1	256·8	238·2	202·6	198·4	162·4
	Shoot	198·4	175·8	162·7	142·4	129·8	102·4	98·2	78·9
	Total	574·2	486·2	460·8	399·2	368·0	305·0	296·6	241·3
	Per mg Wt	287·1	243·1	230·4	199·6	184·0	152·5	148·3	120·65
TER	Root	275·8	236·8	214·1	190·4	169·9	152·4	137·1	124·1
	Shoot	152·4	128·4	108·5	97·1	80·2	60·4	59·0	40·0
	Total	428·2	365·2	322·6	287·5	250·1	212·8	196·1	164·1
	Per mg Wt	214·1	182·6	161·3	143·75	125·05	106·4	98·05	82·05
Rate of synthesis (%)		74·57	75·11	70·01	72·02	67·96	69·77	66·12	68·01
TER TDH × 100									

Values are average (±0·001 − 0·01) of 6 replicates.
UT, No phenol; PT, phenol treated.

$$RPi = \frac{P_f - P_i}{P_i \times \text{post inoculation period (45 days)}}.$$

P_f is the final pathogen population and P_i is the initial inoculum applied.

Triphenyl tetrazolium chloride (TTC) was employed as the artificial electron acceptor for determining the redox enzyme activities. The rates of synthesis in the plants were then computed as a measure of relations between the total dehydrogenases (TDH) and the total endogenous reductases activities (TER) (table 2) (Kannan 1967, 1968).

3. Observations

Table 1 shows that for the initial inoculum (P_i) of 10^3 juveniles, the 4 viable genomes exhibit varied amount of final population (P_f) at the end of 45 days, indicating their susceptibility ratings (SR). The SR in them runs in a decreasing order as follows: *H. canabinus* > *P. mungo* > *H. esculentus* > *S. melongena*, indicating the high susceptibility of *H. canabinus* and low susceptibility of *S. melongena* under NPK influence.

The introduction of phenol resulted in a smaller size of the final population, reflecting the alleviating effect of phenol by reducing the pathogen impact, though of course in accordance with the susceptibility ratings of those hosts. The final population load in the 4 viable hosts under NPK and under the influence of phenol, indicate the host responses in this screening test.

In contrast to the hosts' susceptibility ratings, the total ERF-(table 1) encountered by the pathogen in its two environs, viz. inside the plant and outside the soil, is observed to operate in the reverse direction of the hosts' susceptibility ratings, as given here: ERF = *S. melongena* > *H. esculentus* > *P. mungo* > *H. canabinus*. Thus a host with high SR as *H. canabinus* is associated with low ERF and *S. melongena* with low SR, is associated with high ERF, both under NPK and under phenol influences.

Host pathogen relations (HPR) is the resultant of 2 forces viz. plant and the pathogen, both of them subjected to the soil influences. It can be observed that success of the pathogen is the result of a low resistance encountered by it in the plant environment and also the soil, which is finally exhibited as high or low pathogen impact on the host, which is now graded genetically for its SR or resistance as the case may be. Therefore it is clear that the final interpretations of the host-pathogen relations are based on the interactions of the 2 forces viz. SR and ERF. Hence HPR can be signified as SR↔ERF or HPR = SR/ERF indicating the role of the host and pathogenic factors.

These factors are applicable to both the NPK and phenol treatments as indicated by the varying pathogen loads (P_f) sustained in the 4 viable hosts, indicative of the SR and the varied ERF observed in the plant and in the soil (table 1). The influence of the environmental resistance over the pathogen, inside the plant and outside the soil, is well observed in the varied pathogen contents in these 2 environs and the specific influence of phenol is well observed (table 1) in the increased ERF in both the environs as a result of which the final pathogen load is depressed in these 2 environs, resulting now in altered HPR relations in the 4 viable genomes, which are now presumed to exhibit improved vigour through phenol amendments of the soil.

It is well known (Van Gundy and Stolzy 1961; Van Gundy *et al* 1964; Kirkpatrick

et al 1964; Dropkin 1969; Wallace 1969; Nardicci and Barket 1979), that, extremes of soil environment (soil heat, porosity, moisture, pH and O_2 relations) which impede the plant growth also impede the pathogen's success and that, a well nourished plant rallies round the infection by virtue of its nutrition. Thus the successful pathogenesis has the environmental bearing also, in addition to the host's genetic viability or otherwise.

It is therefore clear that breeding for resistant hosts, involving gene shuffle and soil amendments, are ultimately meant to pose altered environs to the pathogen, to contain its impact, if not totally eradicate that. The utility of soil amendments is centred around the environmental impacts on the pathogen, more possible and easily, by varied amendments with different agents as phenols, phenolics etc. since in genetic reshuffle it is not possible to dictate the polygenic interplay, while at the same time the evolution of resistant biotypes of pathogens cannot also be ignored, for a given genetic effort.

The source size, strength-sink size and strength (Cowling and Horsfall 1980) show the bidirectional transit of raw and finished products through the metabolic continuum in the plant. It is also known (Cowling and Horsfall 1980) that during crisis (dietary and pathogenic) the host spends the minimal energy to meet the crisis demands, conserving the rest for repair through synthesis of proteins, lipids, phenols etc. through metabolic pathways involving redox enzymes (Goodman *et al* 1967). It is needless to point out such synthesis during nematosis as extensively documented in nematology.

The quientisence of the above is reflected in table 2, indicating the redox metabolism during the pathogenic crisis under NPK and phenol influences. It is observed that the dehydrogenases are more active in a highly susceptible host as *H. canabinus*, than in *S. melongena* in conjunction with their SR ratings, under NPK treatments. Their recoveries through endogenous reductases also run in the same fashion. With the introduction of phenol, which resulted in improved ERF values and hence lowered pathogen population, the dehydrogenase activities also fall down, indicating reduced catalysis due to reduced pathogenic impact. Correspondingly the synthetic rates as expressed by endogenous reductases, are also depressed, suiting the need of the demands.

The metabolic continuum in the plant is known (Cowling and Horsfall 1980) to compensate for the ill effects of crisis (dietary-pathogenic) by reallocation of resources, triggering reactions at all regions, which exhibit different responses of growth and metabolism, which ultimately are involved in compensations through growth and metabolism. Such compensations are also observed in the present studies in the differential redox metabolic responses of the root and shoot under NPK and phenol influences. A significant feature in the present studies is, the significant recovery gains effected ranging from 66–75% under various nutritional impacts as observed in table 2, indicating the efforts put up by the viable genomes to tide over the crisis. In otherwords, such viable genomes with genetically ingrained susceptibility, exhibit functional resistance, brought to play during the crisis, which is now interpreted in terms of improved vigour of those susceptible hosts.

Such an improved situation is due to phenol's high compatibility with the host's factors (metabolites) and its repressive effects on the pathogen's enzyme. Posed with these two hazards, viz. altered internal milleau of the plant and enzyme repression, this obligate endopathogen is faced with impeded nutrition with consequent impact

on its progeny and hence reduced pathogenic impact on the host, which is interpreted in terms of improved vigour of the viable host.

Therefore, agents like phenol exhibiting metabolic compatibility with host and pathogen factors, can be advantageously exploited for improving plant vigour for better agricultural gains. Such agents, if with extensive side radicals of high reactive nature, permitting high dilutions as phenol in the present case, require exploration since the high dilution factor will help reduce the cost of control measures.

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On the improved vigour of the root–knot nematode infected cowpea plant with phenols of palmyrah wood sawdust extract, fortified with gibberellic acid and indoleacetic acid

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Abstract. With NPK solution as the basic nutrient applied to the root–knot nematode infected cowpea plants, amendments were made with 100 ppm of phenol prepared from alcoholic extract of palmyrah wood sawdust. Further amendments consisted in adding gibberellic acid or indole acetic acid or gibberellic acid + indole acetic acid. Amended nutrient applications reduced galling, fecundity of the pathogen, pathogenic impact, improved root weights and synthesis of metabolites which signified the improved vigour of the infected host.

Keywords. *Meloidogyne incognita*; *Vigna unguiculata*; sawdust extract; gibberellic acid; indole acetic acid; vigour.

1. Introduction

While the various enzymes in plant parasitic nematodes account for the tissue lysis (Roy 1979, 1980, 1981), many of the breakdown products like phenols, uronic acid complexes and disaccharides exhibit compatibility with the host plant tissue metabolites and also with the nematode's enzymes, resulting in altered substrate relations and catalytic repression of the nematode's enzyme (Feldman and Hanks 1971; Giebel 1974, 1982; McIntyre 1980; Wallace 1973), such that the feeding and subsequent progeny output of nematode are lowered, with consequent lesser pathogenic impact. This is finally reflected as tolerance in the susceptible plants. These observations led to the reckoning of phenols, proteins, etc. as the possible mechanisms involved in resistance during nematode infections in plants (Giebel 1974, 1982). At the same time accumulation of (indole acetic acid, IAA) indole compounds (Balasubramanian and Rangaswami 1962; Setty and Wheeler 1968) had also been suspected to be one of the possible reasons for galling in root–knot nematode infections. The higher level of IAA in the galled zones and the normal tissue differentiation adjacent to the galls and the varied influences of IAA and gibberellic acid (GA) in plant pathogenesis (Goodman *et al* 1967) reflect the active IAA-GA relations in the pathological metabolism. While breeding for nematode resistant crops is a welcome feature, resistance breaking biotypes of nematode do also evolve and hence the problem goes unabated. Towards containing the infection, attempts are being made in improving the vigour/tolerance of the susceptible hosts.

 Singh and Sitaramaiah (1967, 1971) stated that the nemastatic factors attributable to sawdust as organic amendment were due to the phenolics released from sawdust. McIntyre (1980) suggested that the phenols can condense with proteins of the substrate and also with the enzymes of nematode, leading to the altered situation of

starved nematodes, and hence lesser catalysis and better performance by the plant and reflected as improved tolerance.

In our laboratory, free phenol in varying dilutions in water, inhibited the hatching of *Meloidogyne incognita* eggs. As a followup, it was observed that in 4 *Meloidogyne* susceptible hosts, phenol at 100 ppm in 2% NPK solutions reduced the pathogenic impact, with altered fecundity rates of the nematode, ultimately resulting in improved performances in those hosts. Further, in one susceptible host (cowpea), whereas 1:1000 dilution did not appreciably retard the pathogenic impact, and whereas 1:10 retarded the plant growth, 1:100 dilution yielding 100 ppm of phenol checked the infection, without affecting the host plant. In view of the last observation, experiments were conducted to assess the efficacy of phenols from the alcohol extract of palmyrah wood sawdust, as well as the influence of hormone and auxin, viz. GA and IAA on the root–knot nematode pathogenesis in cowpea.

2. Materials and methods

Seeds of the cowpea (*Vigna unguiculata*) were surface sterilized with 0·1% $HgCl_2$ washed with sterile water and sown in 10 cm diameter pots containing river sand only, to eliminate the influence of intrinsic phenol factors in the garden soil. Thirty pots were filled with sand, sterilized at 20 lbs pressure for 2 h and cooled. The soils were irrigated with 1% NPK (commercial) fertilizer solution. This basic nutrient was later amended with phenol, GA or IAA or both. Each plant was inoculated at 2 leaf stage with 1100 infective juveniles of root–knot nematode, *M. incognita*. Fifty ml of the amended nutrients were applied twice weekly with intermittent NPK wash.

2.1 *Treatments*

The following 6 treatments were introduced in 5 replicates

(i) CUT	Control (uninoculated with nematode), untreated (1% NPK solution only, basic nutrient).	
(ii) IUT	Infected (inoculated with nematode), untreated.	
(iii) IT	Infected, treated (100 ppm phenol per 50 ml of basic nutrient).	
(iv) IT	Infected, treated + GA − 0·05 mg.	
(v) IT_2	Infected, treated + IAA − 0·05 mg.	
(vi) IT_3	Infected, treated + (IAA + GA) − 0·10 mg.	

Seventyfive g of palmyrah wood sawdust were refluxed for 1 h with 375 ml of absolute alcohol, filtered and the filtrate used as source of phenol.

Plants were allowed to stand for 30 days, after which the assay of the pathogen's population, reproductive rate etc and the biochemical parameters of the host such as sugars (Seifter *et al* 1950), lipids (Bragdon 1951), proteins (Lowry *et al* 1951), phenols (Bray and Thorpe 1954) and redox-enzymes (Kannan 1967a, b, 1968) were investigated.

2.2 *Nematode population*

The following parameters were employed for evaluation:

(i) Rate of reproduction $(RR) = \dfrac{(P_f/g \text{ root wt})/\text{female}/g \text{ root wt}}{1 \times 100}$

(ii) Rate of population increase (RPI)

$$= \frac{P_f - P_i}{P_i \times \text{post inoculation periods (30 days)}}$$

(iii) Environmental resistance factor (encountered by the nematode) (ERF) = RR/RPI.

P_f is the final nematode population which includes eggs × eggmasses + juveniles in the plant and the soil after 30 days of infection and P_i is the initial inoculum of nematode. ♀ per g weight = total number of ♀ required to produce the P_f based on eggs per eggmass assuming that each eggmass is the product of one female developed from one juvenile. The soil population was estimated by screening 100 g aliquots of soil through a meshset and counting. The population in the plant was estimated by blending 10 g aliquots of shredded infected roots, followed by screening and counting of the larvae and also the eggs in the egg masses.

3. Results and Discussion

Statistical analysis of critical difference at 5 and 1% indicated that the growth (weight of the root tissue) and biochemical parameter of the host plant (*V. unguiculata*) and population buildup of *M. incognita* were more significantly influenced by all the treatments.

3.1 *Pathogenic impact*

In the infected plant (IUT) receiving the NPK solution only as the basic nutrient, a progressive increase in the root weight was observed when phenol, GA, IAA, GA + IAA were introduced (table 1). Among the amending factors GA + IAA with phenol resulted in the highest increase. Contrary to the increase in root weight, galling was reduced with phenol introduction, the reduction occurring further and further as GA, IAA or GA + IAA were employed along with phenol (table 1). Thus GA and IAA separately and also together with phenol reduced root-knot index and the galling phenomenon. The reduction in the galling can be linked with the nematodes population turnover.

The significant impact of phenol + GA + IAA (treatment IT$_3$) consisted in the following, as evaluated against the infected plant which received only the NPK (treatment IUT). In IT$_3$, there was significant reduction in galls to 1/6, eggmass to 1/5, eggs per eggmass to 1/7, root and soil population to 1/5, over that obtained in IUT. The reproductive rate depressed from 3·65 (IUT) to 2·47 and rate of population increase depressed from 0·4521 (IUT) to 0·0462 in IT$_3$, indicating a 1/9th value only in IT$_3$ as against IUT. The ERF increased from 8·073 in IUT to 53·579 in IT$_3$, i.e. more than 6 times in the presence of phenol and GA + IAA.

It was therefore evident that, while phenol alone exerted a depressive effect on the nematode, introduction of GA or IAA or GA + IAA exerted further depression by drastically reducing the nematode's fecundity, root-knot index and galling phenomena. Consequently the root weight also increased.

Since the nematode's fecundity follows feeding, the influence of the host environs

Table 1. Dynamics of the root-knot nematode population (*M. incognita*) in cowpea plant (*V. unguiculata*) subject to nutrient amendments.

	IUT	IT	IT$_1$	IT$_2$	IT$_3$	CD 5%	CD 1%
Root weight (g)	1·253±0·038 (−37·54)[a]	1·450±0·045 (+15·72)	1·600±0·074 (+27·69)	1·875±0·023 (+49·64)	2·125±0·036 (+69·59)	0·061	0·083
Root knot index	1·60±0·01	1·04±0·03 (−35·00)	0·58±0·01 (−63·75)	0·32±0·01 (−80·00)	0·16±0·01 (−90·00)	0·02	0·03
Galls/plant	13·00±2·12	10·00±1·41 (−23·08)	6·00±0·71 (−53·85)	4·00±1·41 (−69·23)	2·00±0·71 (−84·62)	1·82	2·48
Egg masses	35·00±2·12	23·00±1·41 (−34·29)	14·00±2·12 (−60·00)	10·00±0·71 (−71·43)	5·00±1·41 (−85·71)	2·17	2·96
Eggs*	3·8451±0·0002 (7000)[b]	3·6628±0·0005 (−4·74) (4600)[b]	3·4472±0·0010 (−10·35) (2800)[b]	3·3010±0·0002 (−14·15) (2000)[b]	3·0000±0·0006 (−21·98) (1000)[b]	0·0008	0·0011
Root population*	3·7688±0·0002 (5872)[b]	3·5906±0·0004 (−4·73) (3896)[b]	3·3979±0·0005 (−9·84) (2500)[b]	3·1772±0·0006 (−15·70) (1504)[b]	3·0107±0·0006 (−20·12) (1025)[b]	0·0006	0·0009
Soil population*	3·4978±0·0008 (3146)[b]	3·3230±0·0001 (−5·00) (2104)[b]	3·1761±0·0018 (−9·20) (1500)[b]	3·1471±0·0011 (−10·03) (1403)[b]	2·7781±0·0036 (−20·58) (600)[b]	0·0025	0·0034
Total population*	4·2046±0·0003 (16018)[b]	4·0253±0·0004 (−4·26) (10600)[b]	3·8325±0·0010 (−8·85) (6800)[b]	3·6908±0·0006 (−12·22) (4907)[b]	3·4191±0·0013 (−18·68) (2625)[b]	0·0011	0·0014
RR	3·650±0·007	3·178±0·004 (−12·93)	3·040±0·007 (−16·71)	2·618±0·004 (−28·27)	2·470±0·007 (−32·33)	0·008	0·011
RPI	0·4521±0·0003	0·2879±0·0003 (−36·52)	0·1727±0·0005 (−61·80)	0·1154±0·0002 (−74·48)	0·0462±0·0002 (−89·78)	0·0030	0·0040
ERF	8·073±0·010	11·033±0·022 (+36·67)	17·601±0·007 (+118·02)	22·708±0·035 (+181·28)	53·579±0·195 (+563·68)	0·118	0·161

Each value (mean ± SD) represents an average of 5 observations.
CUT root weight (g): 2·006±0·073.
Numbers in parentheses indicate per cent over IUT or *CUT. [a]Log transformed value. [b]Original value.

are well reflected. A progressive increase in sugars, lipids, proteins, phenols and redox-enzyme activities was found, indicating the dynamics of pathological metabolism of the infected plants under various nutrient treatments, with the control checks as the standard for evaluation of such events (CUT and IUT, table 2).

While the sugar reduction, common in root–knot nematode infections, are reflected in the infected plant under NPK influence only (CUT and IUT), sugar levels increasing with phenol application to start with, gradually showing further increments with GA, IAA, GA + IAA applications (highest in IT_3). This indicate the buildup of carbon reserves as sugars etc. under these amendments and also perhaps their depressed consumption by the nematode in the presence of phenol which is believed to cause enzyme repression in the nematode's enzyme.

The biological organization shows a preferential utilisation of proteins and sugars for energy transduction (Fruton and Simmonds 1939; West *et al* 1968) so that, these are utilized faster than fats which serve as final endogenous energy store. It is quite likely that, the impediments caused by phenol in condensing with the plant's metabolites such as glycosides and proteins etc. reduce the readily available energy in sugars for the nematodes. Enzyme repression in nematode can also be brought about by the phenol and this might be responsible for the retarded nematode fecundity in phenol treatment to plants. Further, the high level of protein might be another factor, perhaps not wholly conducive for the nematode.

Coupled with the above are the defence attributes attached to proteins and phenols, in the plant's resistance during pathogenesis, as well as the high energy play reflected in the enhanced redox-enzyme activities (Goodman *et al* 1967).

A perusal of the redox-enzyme activities shows a specific increase of dehydro-

Table 2. Biochemical components and enzyme activities in *V. unguiculata* under root–knot nematode infection and amendments with nutrients.

	Sugar (mg/g)	Protein (mg/g)	Lipid (mg/g)	Phenol (mg/g)	Total dehydrogenase activities	Total endogenous reductase activities
					(mg TTC reduced/g dry weight)	
CUT	6·21 ± 0·08	3·05 ± 0·04	80·19 ± 0·42	3·01 ± 0·01	6·638 ± 0·179	21·070 ± 0·778
Infected plants with various nutrient treatments						
IUT	2·58 ± 0·03 (−58·45)*	3·50 ± 0·06 (+14·75)*	96·56 ± 9·15 (+20·41)*	4·08 ± 0·05 (+35·55)*	7·978 ± 0·382 (+20·19)*	22·270 ± 0·919 (+5·70)*
IT	4·25 ± 1·25 (+64·73)	4·89 ± 0·41 (+39·71)	135·11 ± 18·40 (+39·92)	6·09 ± 0·06 (+49·26)	12·818 ± 0·336 (+60·67)	23·600 ± 0·990 (+5·97)
IT₁	5·63 ± 0·88 (+118·22)	6·14 ± 0·10 (+75·43)	149·23 ± 3·64 (+54·55)	7·22 ± 0·74 (+76·96)	17·568 ± 0·983 (+120·21)	28·670 ± 1·485 (+28·74)
IT₂	7·06 ± 0·91 (+173·64)	7·54 ± 0·42 (+115·43)	162·40 ± 1·71 (+68·19)	8·88 ± 1·61 (+117·65)	20·645 ± 1·229 (+158·77)	33·500 ± 1·980 (+50·43)
IT₃	10·20 ± 0·15 (+295·35)	9·05 ± 0·12 (+158·57)	188·73 ± 2·15 (+95·45)	9·79 ± 0·56 (+139·95)	23·793 ± 1·423 (+198·23)	44·970 ± 3·536 (+101·93)
CD 5%	1·05	0·36	12·42	1·10	1·287	2·557
CD 1%	1·43	0·49	16·94	1·49	1·756	3·487

Each value (mean ± SD) represents an average of 5 observations.
Numbers in parentheses indicate per cent over IUT or *CUT.

genase activities in the infected host under the NPK and other amendments, being highest in the plant, treated with phenol + GA + IAA. This indicates greater energy yield through catalysis, due to expected increase in tissue damage by nematode. However, this seems to be offset by the increased velocities of endogenous reductases involved in synthesis. In fact, the synthetic velocities of enzymes are far more pronounced than the velocities of catalytic dehydrogenases, amounting to almost 1·5–2 times greater than the lytic enzymes i.e. dehydrogenases. The resultant of these two enzymes activities is quite evidenced in the increments of sugars, lipids, proteins and phenols, when phenol or other factors like GA, IAA alone or coupled are introduced as treatments to plants. It is quite likely that the preponderance of energy produced due to catalysis, is transducted towards significant synthetic activity, meant for repair and maintenance through the reductase play.

The above features amplify well the observations of Cowling and Horsfall (1980) who stated that, stress (dietary/pathogenic) in plants is countered through the metabolic continuum through which reallocation of resources occur to combat the crisis and in this direction, the host plant spends the minimal energy for the combat, conserving the rest for repair and growth. That this is so, can well be understood when the redox-enzyme activities are computed against the pronounced synthesis of metabolites during infection. As a net effect, improved tolerance or vigour is reflected, especially under amended conditions, such as introduction of phenol etc. which have a metabolic role in the plant, with the enhanced levels of the various metabolites.

If tolerance can be interpreted as that signifying the capacity of the host to get loaded heavily with pathogen but yet survive, the specific reduction of pathogen's populations with improved weights in the host, signify improved vigour simulating resistance relations as a result of development of post infection resistance, reflecting functional resistance. The reduced galling phenomenon, coupled with the improved weight of the roots and the enhanced metabolism observed in the present studies seem to indicate improved vigour of the host under the influence of phenol + hormone + auxin. Nemastatic factors like phenols in the sawdust extract preparations, produced out of high dilutions and coupled with minute levels of hormone and auxin, thus, can be advantageously exploited for improving the vigour of viable hosts for better produce. The high dilution factor with low levels of hormone and auxin, also help reduce the cost of application, quite favourable for agricultural economy.

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Chemical analysis of secretion from the abdominal scent glands of *Chrysocoris purpureus* (Heteroptera: Pentatomidae)

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Abstract. The chemical analysis of the abdominal scent glands of *Chrysocoris purpureus* Westw. showed the presence of *trans* hex-2-enal, n-dodecane, *trans* hex-2-enyl butyrate and n-octyl acetate. The scent components are metabolically synthesized within the cells of exocrine glands of bugs and are used as defensive in nature. They do not show any correlation with the secondary metabolites of the host plant.

Keywords. Scent glands; *Chrysocoris purpureus*; aliphatic compounds; secondary metabolites; defensive.

1. Introduction

Certain heteropteran bugs on being disturbed, discharge an offensive odour from the scent glands. These glands are situated ventrally in the metathorax of adults and dorsally in the abdomen of nymphs. Morphology and functional anatomy of the scent glands of pentatomid bugs have been studied and the chemical analysis of the secretions of these glands showed saturated and unsaturated compounds (Remold 1963). Many authors have studied the abdominal scent glands of various bugs (Stein 1967; Levinson *et al* 1974; Aldrich *et al* 1972) and their secretions have been identified (Waterhouse and Gilby 1964; Baggini *et al* 1966; Staddon and Olagbemiro 1984; Janaiah *et al* 1979; Leela Kumari *et al* 1984). The chemicals employed by insects to repel predators would be either synthesized by the insects (Meinwald *et al* 1966; Happ *et al* 1966) or sequestered all or half part in host plants (Rothschild 1970; Rothschild *et al* 1970). The present communication deals with the chemical analysis of abdominal scent glands of *Chrysocoris purpureus* and an attempt has been made to correlate the secondary metabolites of the host plant and the components of the scent.

2. Materials and methods

The nymphs of *C. purpureus* Westw. were collected from *Croton sparsiflorus* Morong (Fam. Euphorbiaceae) near the University campus. The nymphs were maintained in the laboratory, on the host plant leaves at room temperature for one or two weeks. The secretion was collected from the abdominal scent glands of 50 bugs inserting microcapillaries against the openings of abdominal glands. The collected scent was injected into gas-liquid chromatography (GLC) and comparison was made with the authentic samples.

2.1 *GLC*

Hewlett Packard 5840-A gas chromatograph was equipped with thermal conducti-

vity detector. Nitrogen as a carrier gas was introduced at 45 ml/min (2·6 kg/ regulator) stationary phase 6 ft × 1/s; packed with 5% silicon (SE)—30 chromosorb P (40–60) mesh at an oven temperature of 100–200°C programming at 5°/min.

The samples (unknown) of 1·5–6 μl were injected at sensitivity 2 and in case of reference (authentic) compounds (ICN K and K Laboratories, New York) 1 μl was injected at sensitivity 16. The scent liquid of *C. purpureus* was dissolved in chloroform and used.

2.2 *Extraction of secondary metabolites from host plant*

The presence of secondary metabolites from leaves and inflorescence of *C. sparsiflorus* were determined by alkaloid test.

The green leaves and inflorescence of *C. sparsiflorus* were collected and dried in the shade and fully dried leaves and inflorescence were powdered. 20 g of the powder was weighed and packed in the filter paper and extracted in a Soxhlet with petroleum-ether (BP 60–80°C), benzene, acetone and alcohol in succession. The extracts were concentrated and the residue dried in a vacuum dessicator.

2.3 *Alkaloid test*

The methanolic solution of the alcohol extract with the help of capillary was applied to the silica gel TLC plate and developed in the chloroform: methnol (9 : 1 v/v) solvent in a chromatographic tank. The plate was allowed to dry and it was sprayed with Dragendorff's reagent modified according to Munier (1953). Formation of red-magenta coloured spots indicated the presence of a number of alkaloids in the plant extract.

3. Results

The identification of the scent components was mainly by the comparison with the known standard samples on the GLC. Authentic samples were used for comparison. A study of their retention times (R_t) revealed their identity (table 1). A perusal of table 2 and figure 1 showed the following components.

Peak 1.   The peak with a R_t 2·25 min corresponded to *trans* hex-2-enal.

Peak 2.   The peak which had a R_t 2·50 min corresponded to n-dodecane.

Peak 3.   The component with the R_t 6·25 min corresponded to *trans* hex-2-enyl butyrate.

Peak 4.   The compound which had a R_t 7·30 min corresponded to n-octyl acetate.

### 3.1 *Secondary metabolites*

The presence of secondary metabolites from host plant, *C. sparsiflorus* were determined by alkaloid test. A number of alkaloid spots were detected (figure 2) after spraying them with Dragendorff's reagent.

Table 1. Some authentic samples with retention time on GLC (under standard conditions).

Component	R_t (min)
n-Butyl butyrate	2·0
Hexenal	2·10
n-Hexyl acetate	2·15
Trans hex-2-enal	2·25
n-Dodecane	2·50
n-Hexanol	6·0
Trans hex-2-enyl butyrate	6·25
n-Pentadecane	6·45
Trans hex-2-enyl acetate	7·0
n-Octyl acetate	7·30
Trans oct-2-enal	8·04

Table 2. Composition of scent from the abdominal scent glands of *C. purpureus* (nymph).

Peak number	R_t (min)	Components
1	2·25	*Trans* hex-2-enal
2	2·50	n-Dodecane
3	6·25	*Trans* hex-2-enyl butyrate
4	7·30	n-Octyl acetate
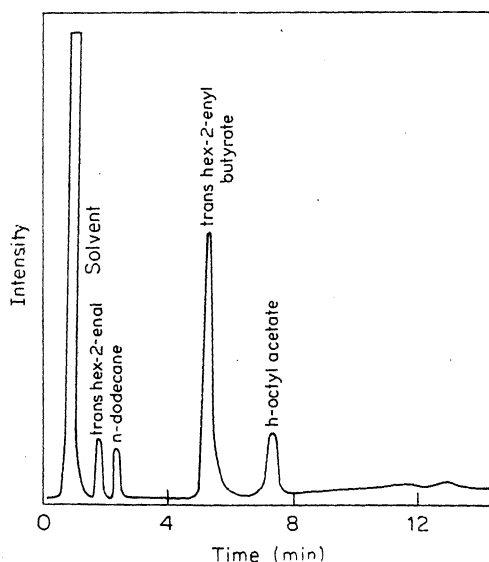
Figure 1. Gas-chromatographic separation of abdominal scent glands secretion of the nymph, *C. purpureus*.

4. Discussion

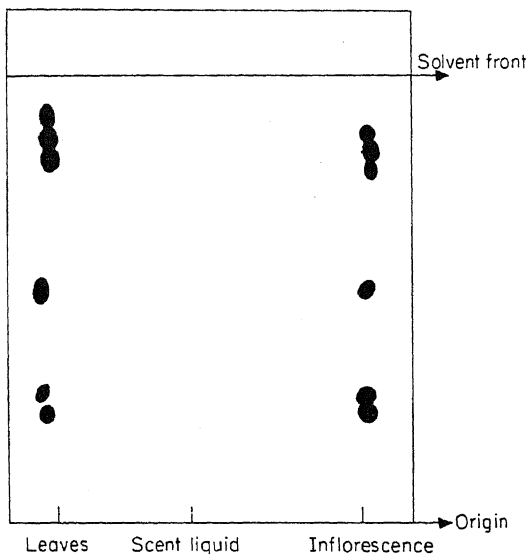Table 2 shows the aliphatic compounds from the secretions of the abdominal scent

Figure 2. TLC separation of alkaloids from the host plant, *C. sparsiflorus* (chloroform: methanol—9 : 1—temp. 38°C).

glands of *C. purpureus*. In general, the unsaturated aliphatic aldehydes and some hydrocarbons have been identified from the secretions of dorsal abdominal scent glands of some members of Heteroptera (Baggini *et al* 1966; Games and Staddon 1973; Baker and Jones 1969). The function of the scent in a majority of insects has not been properly investigated. It was observed that the abdominal scent glands of nymphs of *C. purpureus* yielded 4 components. One of them was shown to be *trans* hex-2-enal which was a common occurrence in nymphs and adults and had an alarm effect to both larvae and adults (Calam and Youdeowei 1968) or non-species-specific alarm effect against the larvae of pentatomid (Ishiwatari 1974). *Trans* hex-2-enyl acetate was found in the abdominal scent glands of the male water bug *Belostoma indica* (Butenandt and Tam 1957) and thought to be a sex-attractant. The nymphs of coreid bugs showed n-octyl acetate and n-dodecane (Aldrich *et al* 1976). The scent from the stink glands of adult *C. purpureus* has also got a defensive property in it. When it fell on the skin it formed blisters with itching in addition to paralyzing small insects (Leela Kumari *et al* 1984).

C. purpureus was found on young leaves and inflorescence and drew its sap from the host plant, *C. sparsiflorus*. The secondary metabolites of this host plant showed the presence of 6 alkaloids. Though the insects sucked their sap from this host plant, no alkaloid was detected in the cells of the scent glands. In this respect, *C. purpureus* differs from certain other insect species such as *Dysdercus plexippus* (Brower *et al* 1968; Brower 1969) and *Oncopeltus fasciatus* (Singh and Rastogi 1970) both of which were found to sequester chemicals present in their stink glands from their host plants during feeding.

Thus it is clear that the chemical composition of the scent of *C. purpureus* metabolically synthesized by the exocrine glandular cells is quite different from that of its host plants and the scent liquid is used as a defensive secretion against predators in nature.

Acknowledgements

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Pollution of the seas around India

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Abstract. The state of marine pollution along the 7000 km long coastline and 2.015×10^6 km^2 exclusive economic zone of India is summarized. The coastal water receives 4.1 km^3 of domestic sewage and 0.41 km^3 of industrial wastes. Nearly 447 million tonnes of oil and its products are transported through the Arabian Sea and Bay of Bengal with the result that some of our coastal areas particularly adjoining the large cities are getting polluted. Increased eutrophication and decrease in dissolved oxygen associated with the generation of toxic hydrogen sulphide have been observed at several places. Heavy metal concentrations are largely within the acceptable limits in water and in biota excepting in a few areas. Organochlorine and pesticides residues have often been found to be high in zooplankton and in the sediments near the confluence of the river and the sea, indicating their land origin. Oil pollution is a chronic problem in the northern Indian Ocean. Several of the endangered ecosystems have now been offered protection by declaring them as marine parks. It is recommended that to maintain coastal waters clean, wise and judicious use of the ocean must form an integral part of our planning.

Keywords. Pollution; sewage; metals; pesticides; oil; estuaries; coastal environment.

1. Introduction

A decade of marine pollution investigations has been completed since the commissioning of the first oceanographic research vessel *Gaveshani* in February 1976. The ocean research vessel *Sagar Kanya*, commissioned in June 1983, has since generated a wealth of information which can be accepted as the state of art documentation on the health of the seas around India. These pioneering efforts have given thrust to marine pollution research in India and through the concerted efforts of the Department of Ocean Development and the National Institute of Oceanography, the programme of work in this discipline is presently well organised. The need for a sustained activity has been clearly spelt-out (Qasim and Sen Gupta 1983; UNEP 1986).

2. Sources of pollution

Johnston (1976) has characterised 3 broad categories of marine pollutants, namely native or natural which are not caused by man, generated by man but not created by him, and the synthetic pollutants wholly created by man. One can broadly put hydrocarbons, soluble inorganic and organic substances in the first category; redistribution and exploitation by man of these hydrocarbons in the second; and plastics, radionuclides and pesticides in the third.

Estimates of the pollutants entering the seas around India made in 1986 are given in table 1. It is clear from this table that a considerable amount of polluting

Table 1. Human pollution and related data together with estimates of pollutants entering the seas around India (as in 1986).

Population	750 million
Coastal population (25% of total)	188 million
Area of the country	$3 \cdot 276 \times 10^6$ km^2
Agricultural area	$1 \cdot 65 \times 10^6$ km^2
Exclusive economic zone	$2 \cdot 015 \times 10^6$ km^2
River runoff (annual mean)	1645 km^3
Rainfall per year (on land)	$4 \cdot 0 \times 10^{12}$ m^3
Rainfall per year (on Bay of Bengal)	$6 \cdot 5 \times 10^{12}$ m^3
Rainfall per year (on Arabian sea)	$6 \cdot 1 \times 10^{12}$ m^3
Domestic sewage added to the sea by coastal population per year (60 l per head/day)	$4 \cdot 1 \times 10^9$ m^3
Industrial effluents added to the sea by coastal industries per year	$0 \cdot 41 \times 10^9$ m^3
Sewage and effluents added by the rivers to the sea per year	50×10^6 m^3
Solid waste and garbage generated by coastal population per year (0·5 kg per head/day)	33×10^6 tonnes
Fertiliser used per year (30·5 kg/ha.yr^{-1})	5×10^6 tonnes
Pesticides used per year (336 g/ha.yr^{-1})	55 000 tonnes
Synthetic detergents used per year	125 000 tonnes

substances enter the Indian seas annually. To these if natural pollution species of atmospheric and weathering origins are added, it is obvious that these waters would need constant attention of marine scientists. In this communication we have discussed the extent of concentration of different categories of pollutants and their influence on the seas around India.

2.1 *Domestic sewage*

Domestic sewage, in small quantities, is known to fertilise the sea and increases the marine productivity. However, over-fertilisation creates stress leading to eutrophication. In the Arabian Sea where, low concentrations of oxygen naturally occur at intermediate depths, the additions of large volumes of sewage alter the oxygen balance in the surface and sub-surface waters thereby jeopardising the survival of living organisms. A recent study (Naqvi 1987) has indicated that the turnover time of the low-oxygenated and denitrified water is limited to only 4 years. This short renewal time of intermediate waters and the short-term variability of the denitrification intensity suggest that the oxygen poor layer of the northern Indian ocean is an unstable time-variable feature which may react quickly to any future climatic and/or environmental perturbations. Hence, a serious concern is expressed that a slight increase in the organic carbon flux due to pollution, and/or a climatic change could create a very significant impact on these intermediate waters turning them completely anoxic.

The consequences of the discharges of sewage and effluents are not very perceptible all along the Indian coast. However, in the nearshore areas of a few metropolitan cities crowded with large industrial complexes, the effects are indeed becoming very perceptible. Sen Gupta and Sankaranarayanan (1975) observed an increase of about 40% (0·82–1·13 μ mol l^{-1}) of phosphate-phosphorous concentra-

tions during 1959–1974 in the nearshore waters of Bombay. More recent observations reveal a concentration of approximately 2 μ mol l^{-1} (Zingde 1985). Dissolved oxygen concentrations also showed a negative trend from 4·71 ml/l in 1959 to near zero in 1983 (Parulekar *et al* 1985). Higher values of phosphate-phosphorous were observed in the nearshore waters of Madras, as compared to the earlier values (Sen Gupta R, unpublished results). Sabnis (1984) estimated 365 million tonnes (MT) as a total volume of all discharges coming from the Bombay city alone every year. A similar estimate of about 396 MT was given by Ghosh *et al* (1973) from the environs of Calcutta city. These figures give an idea of the total volume of domestic sewage, industrial effluents and other wastes, being discharged into the coastal waters of India.

The Mahim bay in the city of Bombay is one more example of heavy contamination. This bay occupies an area of 64 km^2 and is influenced by semi-diurnal tides with a maximum range of 3 m. Sabnis (1984) has given the figures of the annual discharges to this bay which are presented in table 2. The bay receives 64 MT of domestic sewage annually apart from the other types of wastes which are now partially treated. H$_2$S-S concentrations in this bay range from 1·5–98·4 μ mol l^{-1} depending on the state of the tides. Thus an area, which once was a healthy ecosystem with good fisheries, oyster beds, fringing mangroves and inviting the migratory birds has become one of the most polluted regions in the country. This sets aside the much accepted belief held in the early seventies that 'solution to pollution is dilution'. The waters off Cochin, Visakhapatnam and Madras receiving discharges of domestic sewage and other effluents are also gradually facing a situation similar to Mahim bay. Evidently, appropriate actions to restrict the sources of pollution are therefore needed.

2.2 *Heavy metals*

Metal pollution generally goes unnoticed until catastrophic events like the *Minamata* or the *Itai-Itai* diseases occur. These were caused by mercury and cadmium poisoning respectively in Japan. In aquatic environments, metals have been termed as conservative pollutants because once added to the environment, they prevail for

Table 2. Pollutants discharged into the Mahim bay (Bombay) every year (in tonnes).

Dissolved solids	92,619
Chlorides	37,495
BOD	16,480
Suspended solids	15,649
Sulphates	4,791
Nitrogen	2,236
Phosphorus	383
Iron	162
Manganese	32
Zinc	16

ever. These metals cannot be broken down to harmless substances by bacterial action. Most of the metals are, however, present as naturally occurring substances in extremely low concentrations. They are leached or introduced into the aquatic systems as a result of weathering of soils and volcanic rocks and as a result of human activities involving mining and metal industry. These processes and activities change the natural concentrations of metals in the sea water resulting in 10- or even 100-fold increase near the point of an effluent discharge. While manganese, copper, iron and zinc are considered essential micronutrients, mercury, cadmium and lead are not required for any important biological function by organisms and are deemed as non-essential elements.

Metal pollution in the seas around India has not yet reached dangerous levels. But the potential threat it poses is serious enough to merit dependable and appropriate monitoring programmes. Metals introduced in the sea water as contaminants undergo various alterations. Apart from dilution and dispersion, the biogeochemical processes remove metals from the sea water or reduce their concentrations in the sea water. These are precipitation, adsorption on to suspended matter and absorption by the organisms. It is the last process which is of prime concern to man and has rightly created much interest in determining the levels of heavy metals in a wide variety of commercially important marine fishes. It is generally believed that for every metal there is one or more organisms which can bio-accumulate it. Presently, therefore, efforts are being made to identify such organisms which might have potential to emerge as pollution indicators or the survival organisms as they are called.

Efforts are also being made on establishing the baseline levels of several heavy metals, especially those which are toxic like Hg, Cd and Pb in various areas of the marine environment around India. The ranges of concentrations of heavy metals in the sea water, as determined by several workers are presented in table 3. As is evident from table 3, a wide variation exists in the ranges reported and this has been assigned to, firstly the area of sampling and secondly the analytical techniques employed by various workers. The ranges of dissolved heavy metal concentrations have been attributed to river discharges (Qasim and Sen Gupta 1983; Sen Gupta and Qasim 1985a). Holeman (1968) has roughly given the amount of sediments added annually to the seas around India by the rivers as 16×10^8 tonnes. A major portion of these additions settles at the confluences of the rivers with the sea. Most of the heavy metals can be expected to be transported through this route. This has been illustrated by our observations in the estuarine regions of the river Ganga. These observations were made in September, when because of monsoon rains, the fresh-water runoff and consequently the suspended solids are expected to be maximum. Analysis of several heavy metals in the suspended matter by filtering large volumes of water every 3 h, over two tidal cycles, at several stations located in the last 125 km stretch of the river, revealed that 5–9% of these suspended and particulate metals are precipitated in the estuarine region, 45–50% in the mouth region and about 40% would finally flow out to the Bay of Bengal. Similar observations on dissolved metals showed that 85% get into the estuaries and at the mouth of the rivers, and the remaining 15% flow out into the Bay of Bengal (NIO 1986).

An analysis of the metal concentrations in zooplankton, shell fish, small and large fishes representing different levels of the food chain, is given in table 4. The tissue-wise break up of the concentrations is given in table 5. As is evident from the tables, the ranges in concentrations vary widely. Higher concentrations of metals (table 5)

Table 3. Ranges of dissolved heavy metal concentrations (µg/l) in the Indian Ocean determined by various workers.

Source	Cu	Cd	Fe	Mn	Zn	Pb	Ni	Co	Hg (ng/l)
Topping (1969)	0·5-49·1	—	0·1-61·8	0·1-4·6	3·9-19·5	—	—	—	—
Chester and Stoner (1974)	0·2-1·2	0·02-0·14	0·5-3·1	0·07-0·37	0·3-3·0	—	0·3-2·6	—	—
Surface only	1·7-7·9	—	7·2-66·9	—	0·5-42·4	—	0-11·5	0-6·6	13-187
Sen Gupta et al (1978)	—	—	—	—	—	—	—	0-6·7	—
Singbal et al (1978)	1·9-19·9	—	8·5-96	1·8-80	1·2-29·7	—	0-16·3	0-0·02	—
Sanzgiri and Moraes (1979)	0·08-0·48	0·01-0·16	0·15-10	—	0·6-13·8	0·02-0·18	0·18-0·95	—	0-24
Danielsson (1980)	—	—	—	—	—	—	—	—	—
Sanzgiri et al (1979)	22-37·2	—	6·2-131·5	1·8-40·8	2·4-20	—	0-12·2	0-7·9	—
Braganca and Sanzgiri (1980)	1-5	0·15-1·9	2-21·7	1·5-24·7	1·2-12·8	0·25-7·5	0-1	0-1	—
Sanzgiri and Braganca (1981)	0·22-1·2	0·005-0·1	—	—	0·002-0·91	0·05-0·12	—	—	—
Fowler et al (1984)	—	—	—	—	—	—	—	—	4-3

Table 4. Ranges of concentration of some essential and non essential heavy metals (ppm wet weight) in zooplankton, crustaceans, bivalves and in the muscles of certain fishes from the northern Indian Ocean.

Fish	Essential heavy metals						Non essential heavy metals		
	Cu	Fe	Mn	Zn	Ni	Co	Pb	Cd	Mg
Zooplankton	2·0–5·0	35·0–94·0	3·0–7·0	8·0–31·0	0·2–3·0	ND–4·0	1·0–12·6	0·02–5·99	ND
Prawns (6 spp)	3·5–24·0	—	—	—	—	—	1·0	0·2–2·5	ND–0·17
Crabs	0·7–13·5	—	—	—	—	—	1·0–7·88	0·61–1·12	0·004–0·01
Clams	—	4·0–62·0	ND–3·7	ND–21·0	—	—	1·28	1·66	0·06
Oysters	45·0	—	—	—	—	—	1·0	1·36	0·02
Mussels	—	—	—	—	—	—	1·31	1·38	0·09
Flying Fish	0·1–0·7	—	—	—	ND–0·9	0·2–1·3	1·08–5·76	ND–0·65	ND–0·07
Silver Bellies	1·0–16	—	—	—	—	—	1–3·21	0·58–2·11	0·001–0·01
Malabar Anchovies	4·4	—	—	—	—	—	1	0·7	0·01
Sardines (2 spp)	0·03	8·0–10·0	0·2	4·5–6·3	—	0·7–1·1	1	ND–0·62	ND–0·01
Mackerel (2 spp)	1·0–1·3	12·0	0·01	8·0	ND	1·8	1	0·22–1·62	0·01–0·02
Jew Fish (2 spp)	ND–0·8	6·0–8·0	0·3–10·0	4·0–48	ND	0·7–1·1	1–1·14	0·19–0·42	0·006–0·01
Perch (3 spp)	0·2–0·7	6·0–29·0	ND–0·1	3·4–61	0·3–0·5	ND	1	ND–1·47	0·007–0·1
Pilot Fish	0·1–4·9	—	—	—	—	—	1–2·95	ND–0·83	ND–0·02
Scianid (2 spp)	0·1–0·3	—	—	—	—	—	1	0·86–1·36	ND–0·02
Sole	—	—	—	—	—	—	1	0·35	0·01
Pomfret	—	—	—	—	—	—	1	0·73	0·01
Cat Fish	—	—	—	—	—	—	1	0·92	0·06
Trevally (2 spp)	ND–0·7	5·0–11·0	0·1–9·0	2·0–5·0	ND–0·6	ND–1·2	1·02	ND–0·62	0·018–0·08
Grunter	0·36	—	—	—	—	—	1	ND	0·24
Talang	0·4	—	—	—	—	—	2·7	ND	0·36
Tuna (4 spp)	0·3–3·0	7·0–164·0	0·1–7·5	4·0–12·0	ND–4·0	ND–3·2	1–3·3	2·00	0·004–0·22
Dolphin Fish	0·2–1·7	13·0–39·0	ND–3·1	5·0–9·0	0·1–1·2	ND–1·9	1–2·95	0·95	0·01–0·14
Seer Fish	0·4	—	—	—	—	—	1·5	0·25–0·66	0·09–0·11
Barracuda	0·1–0·5	4·0–17·0	0·2–3·1	3·3–5·8	0·1–0·3	0·6–1·9	1	ND–0·28	0·06–0·2
Sea Pike	—	—	—	—	—	—	1·46	ND	0·11
Sharks (4 spp)	0·14–1·1	10·0–57·0	ND–2·0	4·5–12·0	ND–0·3	ND–3·8	1–6·02	ND–0·81	0·02–0·21

ND, Not detectable.
Source: Kureishy (1985)

Table 5. Ranges and average concentrations of some toxic heavy metals (ppm wet weight) in different body parts of fishes from the northern Indian Ocean.

Body parts	Mercury		Cadmium		Lead	
	Range	Average	Range	Average	Range	Average
Muscle	ND–0·36	0·07	ND–3·24	0·59	ND–3·43	1·11
Liver	ND–0·04	0·01	1·2–87·3	20·18	1–17·62	3·8
Gill	ND–0·03	0·016	ND–0·76	0·42	1–7·0	3·14
Heart	ND–0·08	0·026	ND–1·91	0·54	1–3·4	1·36
Kidney	ND–0·04	0·015	0·38–36·69	9·02	1–69·46	8·61
Gonads	ND–0·03	0·015	ND–8·06	1·25	1–4·76	1·36

ND, non detectable.
Source: Kureishy (1985).

are recorded mostly in those tissues which are not normally consumed as food. The concentrations of most of the metals are high in the liver, kidneys, gills and other organs, with the exception of Hg, which tends to accumulate more in the muscles. Generally the indications are that most of these metals get assimilated by fishes in fat soluble forms. We have also noted that zooplankton generally have high concentrations of practically all the metals except Hg.

Surface and subsurface layers of the sea provide further evidence that one of the mechanisms of the addition of metals to the sea is atmospheric transport. However, no evidence is available of the transfer of these metals from zooplankton to fish. A correlation between certain metals and the size of the fish and their stages of maturity was found in some species (Kureishy 1985).

The levels of heavy metals found in several marine organisms, more or less, reflect the natural levels of their concentrations occurring in the sea. On the other hand, in areas such as the coastal waters of the Bombay city, particularly the Thana creek and Mahim bay, the levels of heavy metals in organisms are quite high and this clearly indicates considerable environmental degradation of these areas to the level of making some of the animals taken as human food unfit for consumption (Ganesan *et al* 1980).

Some observations made on the heavy metals from the analysis of the sediments can be summarised as follows: Limited data available for Hg, Cd and Pb in the nearshore sediments, do not show any definite pattern. Though mercury was found in very low concentrations in the nearshore sediments, there were some high values recorded off Bombay. Cadmium and lead show random distribution from non-detectable levels to 80 ppm and 350 ppm respectively. More than 80% of the samples showed Cd and Pb below 1 ppm.

2.3 Pesticides

It is estimated that about 25% of the DDT compounds produced to date might have been already transferred to the sea. It has also been estimated that DDT compounds in the marine biota amount to about 0·1% of its total use. Even such small amounts have produced a significant impact. Effects, such as reproductive failures in sea birds and fish, inhibition of photosynthetic activity among algae are a few examples apart from their transfer into the marine food chain.

Most of the reports on pesticide concentrations in human being as well as in

foodstuffs in India are related to terrestrial environment. Kureishy *et al* (1978) reported values of DDT and its isomers as total DDT (t-DDT) of 0·05–3·21 ppm wet weight in zooplankton from the north-eastern Arabian Sea. Recent observations (Kannan and Sen Gupta 1987) from the same area have shown values of 0·379–1·63 ppm wet weight in zooplankton (figure 1). Concentrations of t-DDT in the waters of the eastern Arabian Sea ranged from 0·06–0·16 ng/l while those in the air over the same area varied from 0·93–10·9 ng m^{-3} (Tanabe and Tatsukawa 1980). These residual levels do suggest that the transfer of the compounds through
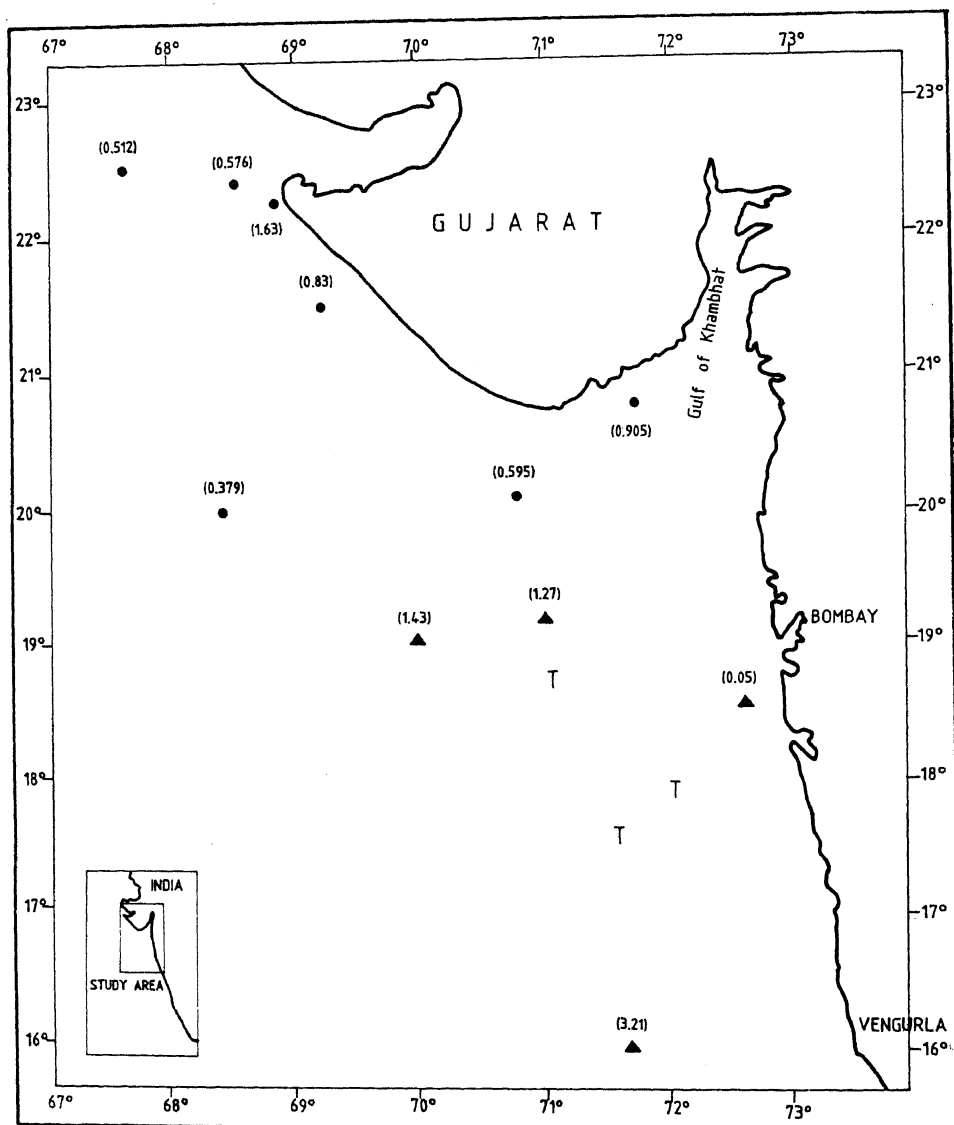
Figure 1. DDT in zooplankton of the eastern Arabian Sea. (▲) Observations made in 1978; (●) Results of observations in 1985. T indicates the presence of organochlorine pesticides residues in trace quantities.

atmosphere to the seas is notable enough to cause concern. The high concentrations of DDT in zooplankton pose a potential threat of their being transferred to higher organisms through food chain. This aspect is receiving attention by workers engaged in environmental monitoring.

A recent study (Sarkar and Sen Gupta 1988) of pesticides residues in the sediments along the east coast of India indicated that apart from DDT and its isomers, residues of gamma-BHC, Aldrine and Dieldrine were recorded from a number of places. Their individual concentrations were, in some areas, higher than t-DDT (table 6). These concentration values are high mainly at the river mouths (figure 2), indicating that these pesticides are of land origin.

2.4 Oil

Sen Gupta and Qasim (1985b) and British Petroleum (1986) reported that the global-marine transport of oil in 1986 was 1264 MT; of which, 447 MT or 35·4% of the total was shipped from the Gulf countries. About two-thirds of this oil is transported across the Arabian Sea to the west and far east along the two tanker routes. The total recorded incidence of accidental oil spills in the Indian Ocean, north of the equator, is 15 tanker disasters and 3 blow-outs from 1970–1982.

The problem of oil entering the marine environment is largely man-made. Oil, once discharged in the marine environment undergoes various biochemical as well as photochemical degradation. Light, volatile fractions evaporate first as the oil film spreads along the water surface and the heavier fractions either get dissolved or start sinking. During sinking, these fractions collect a lot of suspended matter and ultimately settle to the bottom to be later brought up as tar. This tar is subsequently washed ashore on beaches. The effects of discharged oil on the environment are many, depending upon the size of the spill and the area. The most visible effect is on the birds and on the benthic and intertidal fauna. The thin film of oil present at the surface could inhibit the oxygen exchange occurring across the air-sea interface. It can also act as solvent for a wide variety of organic pollutants.

Sen Gupta and Kureishy (1981) reported observations on the oil slicks and other floating pollutants based on the data obtained from the Japan National Oceanographic Data Centre. Out of 6689 observations, about 5582 or 83·5% of the total, showed positive signs of oil slicks or other floating pollutants in the northern Indian Ocean. Figure 3 shows recorded observations made on oil slicks and other floating pollutants over the entire Indian Ocean up to 40°S latitude. The data have been divided in 5° squares. The lower numbers in these squares indicate the occasions when oil slicks were present while those at the top show number of occasions when oil slicks were absent. A close look at the illustrations will indicate that the oil slicks occur very frequently along the tanker routes, while south of the equator there is less oil pollution in the Indian Ocean. Observations on floating tar too revealed a range of 0–6 mg/m^2 with a mean of 0·59 mg/m^2 in the Arabian Sea. The range of the floating tar along the tanker route in the southern Bay of Bengal was 0–69·75 mg/m^2 with a mean of 1·52 mg/m^2 indicating that this area was more polluted than that of the Arabian Sea. It was also observed that the occurrence of floating tar largely depended on the prevailing current patterns, being absent from June–September as the surface currents in the Arabian Sea happened to be towards

Table 6. Organochlorine pesticides residues in the sediments of the east coast of India (μg/g) wet weight.

Lat.°N	Long.°E	Depth (m)	-BHC	Aldrine	O-P'-DDE	P-P'-DDE	Dieldrine	O-P'-ODD	O-P-DDT	P-P'-DDD	P-P-DDT	Total DDT
22°11·2'	88°9·8'	8	0·046	0·315	0·060	0·021	0·046	0·019	ND	ND	0·049	0·149
22°0·4'	88°4·2'	12	0·051	0·456	0·090	0·022	0·513	ND	ND	0·30	0·066	0·478
20°42·1'	88°1·0'	13	0·007	0·059	0·034	0·007	ND	ND	0·352	0·012	0·010	0·415
20°13·8'	88°25·2'	50	0·013	0·044	0·21	0·013	ND	ND	ND	ND	ND	0·223
21°5·7'	88°58·5'	31	0·020	0·089	0·067	0·013	ND	ND	ND	ND	ND	0·080
20°18·1'	87°46'	43	0·015	0·059	0·018	ND	ND	ND	ND	ND	ND	0·018
19°14·8'	85°38·8'	65	0·093	0·065	0·081	0·044	0·058	ND	ND	ND	ND	0·125
19°15·1'	85°31·8'	50	0·045	0·056	0·031	0·024	ND	ND	0·059	ND	ND	0·114
19°15·0'	85°25·4'	45	0·053	0·092	ND	0·046	ND	ND	ND	ND	ND	0·046
19°15·0'	85°25·6'	35	0·079	ND	0·051	ND	0·098	ND	0·042	ND	ND	0·093
19°14·3'	85°18·9'	100	0·208	0·150	0·110	0·083	0·202	0·038	ND	ND	ND	0·231
19°15·3'	85°12·9'	85	0·052	0·040	0·042	0·047	0·154	ND	ND	ND	ND	0·089
19°15·5'	85°8·2'	90	0·056	0·072	ND	0·053	ND	ND	ND	ND	ND	0·053
19°13·8'	85°2·9'	95	0·044	0·051	0·058	0·057	0·177	ND	ND	ND	ND	0·115
19°13·8'	85°59·6'	71	0·104	0·177	0·065	0·068	ND	ND	ND	ND	ND	0·133
17°00·0'	85°00'	75	0·120	0·102	0·119	0·011	0·169	ND	ND	ND	ND	0·130
16°50·2'	82°43·8'	45	0·007	0·028	0·042	ND	ND	ND	ND	ND	ND	0·042
16°26·0'	82°31·0'	40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16°11·0'	81°47·5'	55	0·080	0·107	0·056	ND	ND	ND	ND	ND	0·069	0·056
15°56·5'	81°19·6'	50	0·016	0·080	0·034	ND	ND	ND	ND	ND	ND	0·034
15°39·5'	81°00·0'	48	0·005	0·080	0·023	ND	ND	ND	ND	ND	ND	0·023
15°22·8'	80°33·1'	52	0·016	0·371	0·130	0·015	ND	ND	ND	ND	0·016	0·211
15°0·3'	80°16·7'	52	0·158	ND	0·105	0·018	ND	ND	ND	0·030	ND	0·183
15°0·0'	81°00·0'	80	0·033	0·120	0·076	0·084	0·271	0·029	ND	ND	ND	0·189
13°0·0'	82°0·0'	60	0·030	0·019	0·023	ND	ND	0·018	ND	ND	ND	0·041
13°0·0'	81°0·0'	30	0·026	0·033	ND	ND	ND	0·030	ND	ND	ND	0·030
13°0·0'	80°40'	35	0·034	0·033	ND	ND	ND	ND	ND	ND	ND	ND
13°0·0'	80°25'	45	0·043	ND	0·055	ND	ND	ND	ND	ND	0·133	0·188
12°0·0'	80°56'	19	0·017	0·527	0·103	0·34	ND	ND	ND	0·080	0·197	0·780

ND, Not Detectable.
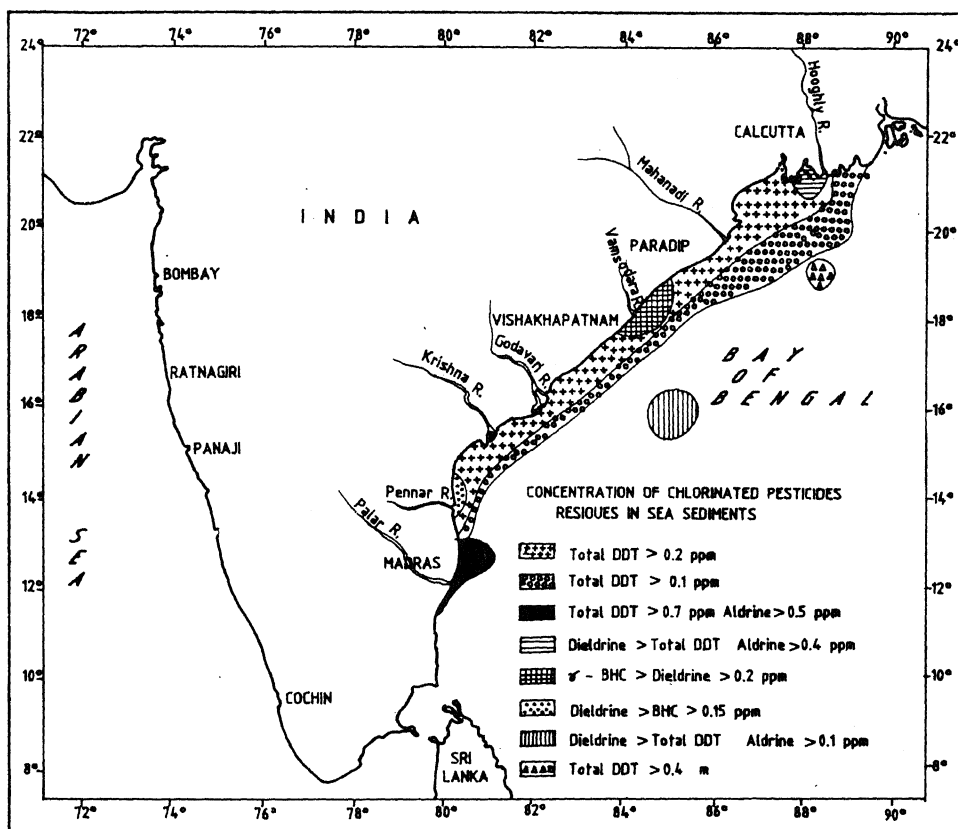
Figure 2. Concentrations of chlorinated pesticides residues in the sediments along the east coast of India.

the Indian coast. The tar particles have a residence time of 30–45 days (Sen Gupta and Qasim 1985b), before they start sinking towards the bottom.

The deposition of tarry lumps on the Indian beaches was first studied in 1975 when it was found that the beaches on the west coast in particular were having a high deposition of tar balls during the south-west monsoon season. This is mainly because the onshore component of the sea currents is strongest from April/May to September/October. Studies in 1975 and 1976 on several beaches of the west coast gave a range of 22–448 g m^{-2} with peak values, on one occasion of 1386 g m^{-2}. As the current pattern reverses, this tar deposition almost stops completely from November–March. The computed total deposit on the beaches along the west coast of India are 1000–750 tonnes for 1975 and 1976 respectively (Dhargalkar *et al* 1977). The concentrations of dissolved and dispersed hydrocarbons in the upper 20 metres are quite uniform in the northern Indian Ocean excepting on a few occasions. These largely depended on the tanker traffic as is evident from table 7.

Oil exploration activities off the Bombay High region are responsible for increased concentrations of petroleum hydrocarbons in the range of 2–46 μg/l in the water and 4–32 μg/g dry weight in the sediments taken from the vicinity of the field. In the same area, after a tanker fire accident, the concentrations of dissolved/dispersed

Figure 3 shows a map of the Indian Ocean region (from 30°N to 40°S, 30°E to 100°E) with country labels including SAUDI ARABIA, IRAN, PAKISTAN, INDIA, NEPAL, BANGLA DESH, BURMA, YEMEN, SOUTH YEMEN, MUSCAT, ETHIOPIA, SOMALIA, KENYA, TANZANIA, MOZAMBIQUE, MALAGASY, and SUMATRA. The grid of 5° squares contains the following recorded values:

		23/16	255/311	310	1/0								
			311/294	380	21	1							
		0/151	464	13	0								
		44	108	85	409	85			11	4			
	25	24/10	94	101	538	115		1/0	0	0			
	96/86	112	118	24	45	439	56	10	12	13	1		
	36	27/82	86	30	59	545	58	7	19	11	0		
	6	142	12	26	34	85	207	432	400	395	319		
	1	64	8	29	44	93	622/524	534	543	403			
	64	88	4	4	4	6	8	14	34	21	167		
	1/10	11	51	3	2	3	5	8	24	40	26	209	
	20	122	10	3	2	5	7	3	22	11	10	13	
	0/1	4	16	0	0	0	0	0	0	0	0	0	
		63	105	30	4	4	4	11	23	2	2	6	4
	1	11	12	0	0	0	0	0	4	0	0	33	2
		133	25	30	4	6	17	18	3	2	3	10	4
		21	0	0	0	0	0	0	0	0	0	0	0
	53/5	85	1	25	30	22	10		3	5	9	5	5
	1	14	0	3	0	0	0		0	0	0	0	0
	124	7	19	38	35	7	2	4	7	1	5	4	6
	0/24	0	0	0	0	0	0	0	22	0	0	0	0
119	58	36	34	9	5	10	13	8	2	6	3	1	2
13	4	0	1	0	0	0	0	0	0	0	0	0	0
128	26	11	16	15	13	9	11	10	8	6	5	3	4
13	0	0	0	0	0	0	0	0	0	0	0	0	0
4	6	2	2	2	2		2		2		2	2	4
0	0	0	0	0	0		0		0		0	0	0

Figure 3. Record of observations of oil slicks in the Indian Ocean from 30° N to 40° S. The values are presented in 5° squares. Numbers at the lower end of the squares indicate the occasions when oil slicks were sighted. Numbers at the upper end indicate occasions when oil slicks were absent.

hydrocarbons went from 27–105 $\mu g/l$ at the surface and from 36–59 $\mu g/l$ at 5 m depth. The concentrations in the sediments increased from 1–26 $\mu g/g$ to 40-512 $\mu g/g$ after the accident.

3. Sensitive environments

Some specialised environments such as estuaries, coral reefs and mangroves are increasingly threatened and deserve special mention as they form spawning grounds and nurseries for a number of important species of economic value. They harbour a

Table 7. Dissolved/dispersed hydrocarbons in the upper 20 m of the Arabian Sea and the Bay of Bengal.

Year	Arabian Sea			Bay of Bengal		
	Transport (Million tonnes)	Concentrations (µg/kg) Range	Mean	Transport (Million tonnes)	Concentration (µg/kg) Range	Mean
1978	975	0·9–42·5	24·31	323	0–28·2	17·14
1979	1010	10·4–41·6	24·48	351		
1980	869	2·4–9·0	5·28	308	1·2–27·4	12·47
1981	725			270	0–4·5	2·25
1982	579			247	0–2·8	1·40
1983	513	0–17·7	5·02	222		
1984	489			252	0–3·4	1·70

wide variety of flora and fauna which can be sources of new drugs and raw materials for several industries.

India has 14 major, 44 medium and 162 small rivers. The mean annual runoff from these rivers is 1645 km³, of which 1600 km³ is the result of rainfall over the catchment areas and 45 km³ is from the melting of the glaciers. A number of dams, barrages and canals in these rivers have been constructed to make their water available for agriculture, power generation and drinking.

Studies conducted on many of the estuaries have shown that most of these are either badly polluted or are getting contaminated mainly by industrial wastes. Almost all of the estuaries are tide-dominated, coastal plain type. Thus a fairly large amount of entrapped waste materials does not easily flow-out from the estuaries into the sea.

The estuaries of the rivers Ganga and Mahanadi have recently been studied for their water quality and recipient characteristics. Some of the physical, chemical and biological features of these estuaries over a period of 3 years have been studied (NIO 1986).

The river Ganga has an annual runoff of 493 km³ and carries 616×10^6 tonnes of suspended solids. Nearly 396×10^6 m³ of waste materials including 122×10^6 m³ of industrial effluents are added annually to the estuarine region of this river. The river is influenced by semi-diurnal tides having a maximum range of 5·5 m during the spring tide. A zone of significant chemical reactions exists in the estuary. There is a clear addition of foreign material to this zone. About 85% of the dissolved metals settle within the river leaving only 15% to flow out. Nearly 10% of the suspended and particulate metals settle within the estuary, 50% at the confluence of the river with the sea and 40% finally flows out to the open sea.

Photosynthetic productivity and chlorophyll *a* decrease downstream. High zooplankton biomass is observed in the estuary, copepods being the dominant group. Numerous larvae and juveniles of commercially important fishes and prawns form another significant component. Microbes such as *Vibrio parahaemolyticus* which is the etiologic agent for food-borne gastroenteritis, are present in the water and sediments of the river mouth. These observations indicate that the water quality of the estuarine region of the river Ganga has deteriorated and needs immediate attention.

The northwest and southeast coasts of the Indian mainland as well as the Lakshadweep and the Andaman and Nicobar groups of islands harbour rich coral reefs and atolls of great beauty. It has been estimated that the lagoons of the Lakshadweep atolls contain upto 2000 MT of calcareous sands. Of this, about 700 MT upto one metre depth could be exploited without affecting the atoll environment adversely (Siddiquie 1985). However, due to oil pollution caused by a heavy tanker traffic in the vicinity and because of collections for ornamental shells and decorative objects and corals, some of the coral reefs on the islands of the Lakshadweep and Andaman and Nicobars are getting badly damaged. An island, in the Gulf of Kutch (Pirotan) has been declared as a nature reserve or marine park. Similarly, the islands of the Gulf of Mannar and Palk Bay in Tamil Nadu have also been declared as protected areas. Another coastal area with coral reefs near Malwan on the central west coast of India is likely to be declared as preserved.

The mangroves too form excellent hatcheries, nurseries and habitats for a wide variety of fish and wildlife. Land reclamation, deforestation for use of timber as firewood, population pressures and pollution are the main hazards for the mangrove environment. As an example, we can cite the case of Mahim creek where industrial wastes and domestic sewage have completely destroyed the once lush green mangroves found all along the creek.

4. Conclusion

Although the extent of pollution in the seas around India is not very severe, it is time we recognise that it needs a sustained scientific effort towards its improvement. The present, not so serious a situation, is largely due to the nature and geographical orientation of the northern Indian Ocean, which is subjected to the impact of semi-diurnal tides associated with the biannual reversal of the direction of monsoon winds and the resulting sea currents. These natural phenomena promote enough flushing and provide adequate exchange of water masses resulting into good dispersal of all the incoming pollution loads. Unlike the Baltic and the Mediterranean seas which are closed and therefore are heavily contaminated due to increasing inputs of land-based pollutants from countries surrounding them, we have the advantage of open seas that ensures good dispersal of the pollutant species. The seas should not be any more considered as dumping ground and this is the time we learn from the experiences of the other countries and evolve appropriate scientific programmes to keep our coastal waters free from pollution.

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Biochemical changes during embryogenesis in *Atractomorpha crenulata* (Fab) (Orthoptera: Insecta)

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Abstract. Quantitative changes in the total proteins, carbohydrates and lipids during embryogenesis of *Atractomorpha crenulata* (Fab) are provided. The protein and carbohydrate contents of the egg increase after 4 days of incubation until just prior to hatching when there is a slight fall. Lipid content shows a steep increase on the 6th day of incubation. Qualitative gel electrophoretic analysis revealed as many as 14 protein fractions at various stages of embryogenesis. These observations are discussed based on densitometric scanning studies.

Keywords. *Atractomorpha crenulata*; biochemical changes; embryogenesis.

1. Introduction

Morphological changes during embryogenesis are closely associated with various aspects of the protein metabolism which involves mainly the break-down of pre-existing yolk reserves and the conversion of these into tissue- and organ-specific proteins (Chen 1966). Hill (1943) studied the carbohydrate metabolism in the isolated grasshopper embryo and reported largest variation in the extra-embryonic part of the egg. There is a steady consumption of both polysaccharides and fats during embryogenesis with the level of glucose lower and more stable than that of glycogen. Qualitative and quantitative changes in the carbohydrates of the eggs of grasshopper *Aulocara ellioti* (Thomas) at various stages of development was investigated by Quickenden (1970). Slifer (1930) observed significant change in the saturation of fatty acids during development of grasshopper eggs. The present communication attempts to study the biochemical changes with reference to the protein, carbohydrates and lipid contents during embryogenesis of *Atractomorpha crenulata* (Fabricius) where no diapause is seen during the egg stage.

2. Materials and methods

Newly moulted adults of *A. crenulata* were separated from the stock culture of acridids reared in wooden cages measuring $25 \times 25 \times 30$ cm and fed *ad libitum* on leaves of *Ricinus communis*. Moist soil was provided in plastic containers for oviposition and observations made daily for ovipositional spots which were tagged. Eggs were collected every 48 h after oviposition until 20 days of incubation i.e. just prior to hatching and stored in deep freeze for analytical studies. Care was taken to use only the first batch of eggs laid by the newly moulted female adults for all biochemical estimations since maternal age has been reported to effect the biochemical composition of the acridid egg (Quickenden and Roemhild 1969). Quantitative biochemical estimation was carried out for the total proteins (Lowry *et al* 1951), carbohydrates (Dubois *et al* 1956) and lipids (Folch *et al* 1957). For the

qualitative profile of the proteins, polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Davis (1964). 7·5% tube gels of length 11 cm were prepared and electrophoresis carried out by adjusting the current to give 2·5 to 3 mA per tube for about 2½ h until the tracer dye migrated to a distance of about 90–95 mm into the running gel. Electrophoresis was performed at 5–10°C. After electrophoresis the proteins were stained with 0·02% Coomassie brilliant blue in a mixture of methanol acetic acid and water (25:7:68). The stained gels were scanned using LKB 2202 ultrascan laser densitometer.

3.   Results

Females of *A. crenulata* fed with *R. communis* lay egg pods in soil which hatch within 20 days under experimental conditions of 30°C and 75% relative humidity. Figure 1 provides the relative changes in the total protein, carbohydrate and lipid contents of the egg during embryogenesis. There is a decrease in the egg weight during the first 4 days of incubation after which there is a progressive increase in weight till day 10. From the 12th day of embryogenesis till nymphal eclosion the weight remains more or less constant. Analysis of the changes in the total protein and carbohydrates indicate a similar trend in that there is no appreciable change in their contents during the early incubation period i.e. until 4th day beyond which there is a steady increase in the total content until just prior to hatching when the protein and carbohydrate levels fall. It is interesting to observe that this increase in the protein and carbohydrate levels correspond to the period when the weight of the egg also increases. The total lipid content shows significant increase at the 6th day of incubation but declines to a low level by the 10th day. Further changes in the lipid content follow a trend similar to that shown by carbohydrates and proteins.

Qualitative analysis of the various proteins that occur during embryonic development of *A. crenulata* revealed interesting patterns of protein metabolism. As many as 14 protein bands were identified through PAGE studies using densitometric scanning techniques (figure 2). Gels were run through a distance of 9·3 cm from the origin and the electrophoretic mobility of the various proteins were measured which enabled
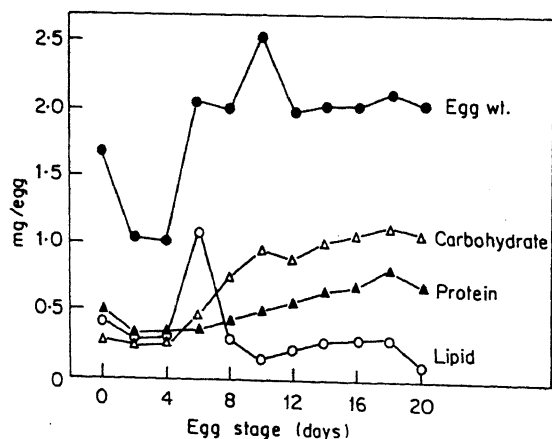
Figure 1. Relative changes in the total proteins, carbohydrates and lipids during embryogenesis.
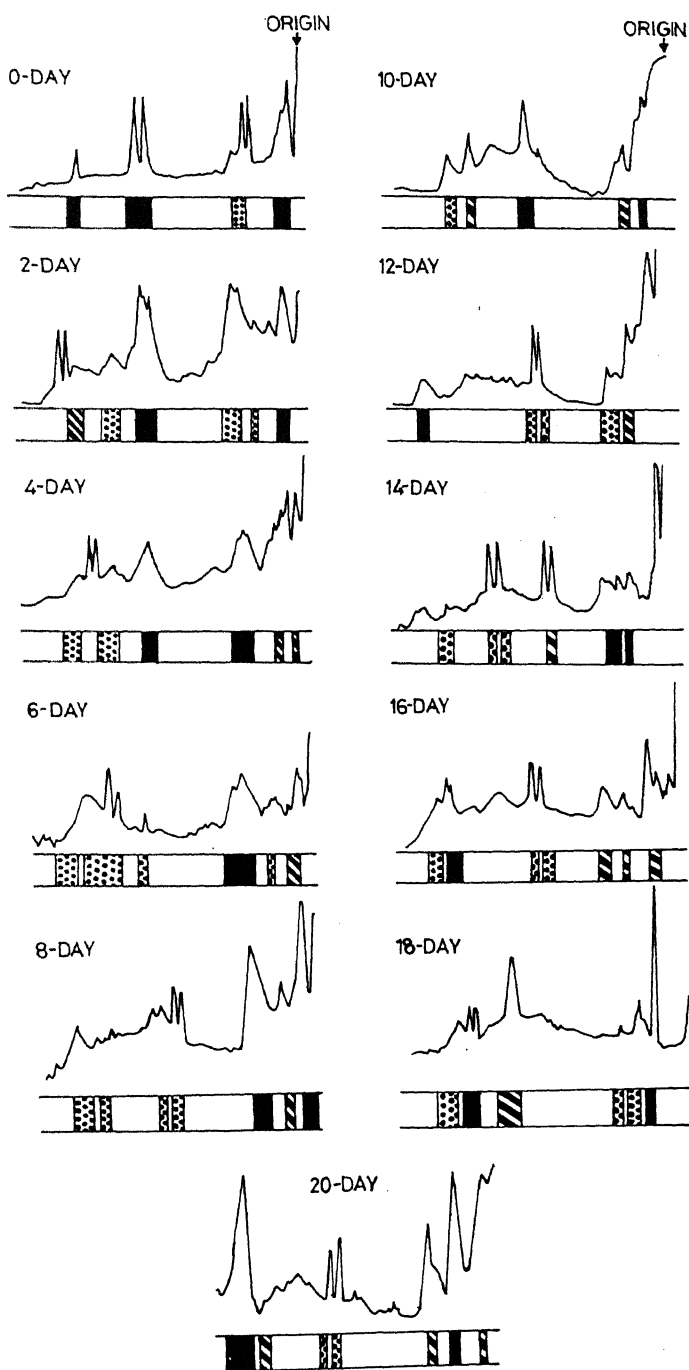
Figure 2. Densitometric scan of protein fractions at various stages of embryogenesis.

comparison of the various fractions through embryonic developmental stages (table 1). Eggs that were just oviposited (0-day eggs) showed 4 distinct bands that had

Table 1. Electrophoretic analysis of the egg proteins of *A. crenulata* during embryogenesis

	Protein fractions													
Egg stage (day) Rm	A (0·075)	B (0·129)	C (0·172)	D (0·258)	E (0·322)	F (0·473)	G (0·505)	H (0·559)	I (0·580)	J (0·634)	K (0·731)	L (0·752)	M (0·838)	N (0·935)
0	44·353 (0·326)	—	—	2·049 (0·020)	—	—	—	32·724 (0·292)	—	—	—	—	20·874 (0·253)	—
2	28·793 (0·258)	—	3·550 (0·050)	—	5·221 (0·250)	—	—	45·412 (0·339)	—	—	3·477 (0·080)	—	13·545 (0·127)	—
4	16·307 (0·202)	13·938 (0·364)	—	35·708 (0·703)	—	—	—	26·855 (0·479)	—	—	3·221 (0·065)	—	3·968 (0·074)	7·175 (0·076)
6	12·799 (0·202)	—	7·736 (0·116)	—	55·810 (0·706)	—	—	9·027 (0·099)	—	—	7·449 (0·095)	—	—	9·755 (0·729)
8	35·979 (0·394)	12·503 (0·333)	—	32·069 (0·541)	—	—	—	2·542 (0·092)	4·574 (0·151)	—	—	—	2·574 (0·116)	—
10	36·372 (0·192)	—	19·032 (0·192)	—	—	—	—	30·497 (0·168)	—	—	13·721 (0·124)	—	0·375 (0·004)	—
12	—	—	12·387 (0·145)	1·259 (0·031)	—	4·827 (0·042)	8·332 (0·059)	—	—	—	—	—	—	73·193 (0·725)
14	—	—	—	39·702 (0·117)	30·509 (0·466)	14·256 (0·122)	—	—	—	—	5·121 (0·055)	3·937 (0·042)	—	6·472 (0·057)
16	—	18·187 (0·114)	—	18·747 (0·254)	19·403 (0·369)	—	4·566 (0·064)	3·635 (0·069)	—	—	—	—	27·276 (0·566)	8·191 (0·133)
18	—	—	45·820 (0·088)	7·743 (0·117)	1·361 (0·089)	—	—	—	1·819 (0·067)	1·777 (0·082)	18·715 (0·148)	—	26·102 (0·446)	1·405 (0·075)
20	17·208 (0·188)	—	22·869 (0·185)	20·500 (0·438)	—	—	—	—	—	—	—	—	11·274 (0·634)	24·548 (0·782)

Values represent area percentage.
Values in parentheses represent area/height.

significantly different electrophoretic mobility namely A-band (0·075 Rm), D-band (0·258 Rm), H-band (0·559 Rm) and M-band (0·838 Rm). Newer protein bands were evident as development of the embryo proceeds. The 2-day old egg showed 6 protein bands of which 3 bands (C, E and K) appear to be formed new. The A-protein band was observed through successive stages of egg development until the 10th day. A critical analysis of the protein bands during the first 8 days of egg development reveal a synchronized occurrence of the D as well as C and E protein bands in every two day succession. It is interesting to note the occurrence of D-protein band consistently from the 12 days egg stage to nymphal eclosion. Of equal significance is the observation of the K-protein band which was evident until the 6th day old stage after which they appear only in the 10th, 14th and 18th day egg stages. It could be generalised that the H and M protein fractions which were originally evident in the 0-day egg do not undergo drastic modification and they appear in almost all the egg stages. However, the occurrence of the N-band during the later stages of embryogenesis is a feature of probable significance in egg development. Proteins corresponding to F, G, I, J and K-bands were only observed as light bands in the gel.

4. Discussion

Morphogenesis and metamorphosis in insects are processes that involve cellular differentiation as well as the assembly of cells into organs, both being influenced by insect hormones. However, cellular differentiation is characterized by the appearance of an end product which is a structural protein (Ilan and Ilan 1973). Therefore fluctuations in the protein profile during embryogenesis is an anticipated fact. The present study on the total proteins, during embryogenesis of *A. crenulata* reveals a tendency to show an increasing trend of variation from the 4th day of incubation which corresponds to a period of 25% incubation. Stay and Coop (1973) observed a similar trend in their study where the proteins were recorded to show a linear increase in developing oocytes upto oviposition. After oviposition, there is no increase in protein until 25% incubation time which is coincident with the onset of increase in dry weight. Protein then continues to rise throughout incubation until just prior to hatching when there is a slight decrease in the rate. A similar trend was also observed in the eggs of *A. crenulata* where the increase in protein content coincided with the stage when the weight of the egg also increased.

Qualitative electrophoretic analysis revealed the presence of 4 protein fractions in *A. crenulata* eggs that were just oviposited. With progressive development of the embryo, newer protein fractions were evident. Since the insect egg is a closed system (Agrell 1964), the newer protein fractions should have arisen through catabolism or through synthesis from already existing free amino acids in the egg. During embryogenesis the concentration of free amino acids at first increase due to rapid breakdown of yolk reserves probably by cethepsin-type enzymes (Kuk-Meiri *et al* 1966). These amino acids are used in the synthesis of proteins in the embryo and the concentration falls as the rate of protein synthesis increases (Chen 1966). Roberts and Smith (1971) observed 26 amino acids in the egg yolk of *Melanoplus sanguinipes*, many of which appeared at different periods during embryonic development. Elliott and Gillott (1979) in their study on *M. sanguinipes* traced the accumulation of proteins from the fat body, where most of yolk proteins are synthesised, via the haemolymph to the oocytes in the ovary. In any case, the developing egg has all the

raw materials required for the synthesis of newer proteins. Certain protein fractions such as the A-band protein, was present only during the early embryonic stages whereas protein fractions D and N were evident during the later embryonic stages. Proteins corresponding to the M-band were present almost throughout embryogenesis. Further studies on the isolation and identification of the protein fractions would throw more light in enhancing our present knowledge.

A decrease in the weight of the egg during the first 4 days of incubation was observed after which there is a drastic increase in weight until the 10th day of incubation. The weight of the egg stabilised to a constant level from the 12th day of incubation till hatching. This fluctuation in the weight of the egg may be due to the uptake of water. Rothstein (1952) observed the water content to increase enormously in the egg during embryogenesis, the uptake of water being mainly from the environment as the metabolically formed water amounts to only a smaller fractions. Eggs of *A. crenulata* being laid in moist soil has .ample scope for absorbing the requisite water from the surrounding environment.

Although embryo lipid increased during development, lipid as a proportion of embryo weight decreases rapidly from oviposition to about 26% of incubation time and decreases less rapidly thereafter. This change in rate occurs when dry weight, protein and carbohydrate begin to increase (Stay and Coop 1973), a feature also observed in the present study. Allais *et al* (1964) observed the decrease in lipid content during embryonic development to be due to the catabolism of glycerides and they also demonstrated lipids to be the major energy source for embryogenesis in *Locusta migratoria*. In view of the fact that there has been a comparative reduction in the total lipid content of *A. crenulata* egg, it could be possible that lipids are the source of energy for the developing embryo from the 8th day of incubation. Also some of the lipids may be broken down for synthesis. However, Bhatt and Krishna (1982) observed an increase in lipid content during egg development of the rice moth *Corcyra cephalonica* which they attributed to be due to phospholipid synthesis during embryo growth.

Changes in the carbohydrate content during embryogenesis of *A. crenulata* is a feature also observed in several grasshopper species (Randall and Derr 1965; Quickenden 1970). Quickenden (1970) opined universal occurrence of trehalose in insect eggs and Bhatt and Krishna (1982) recorded the concentrations of glycogen and trehalose to increase during embryonic development upto 48 h beyond which the level of glycogen alone showed a slight fall. They presumed the initial rise in the proportion of both the saccharides to be a sequel to the utilization of alternate sources of energy during embryogenesis while the reduction in amount of glycogen during the advanced egg stage to be due to its mobilisation in the metabolic cycle for energy supply to the growing embryo. In the present study the decrease in carbohydrate content was observed only in the later stage i.e. just prior to hatching indicating that these also may be utilized as a probable energy source.

Acknowledgements

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Overgrowth competitions amongst encrusting cheilostomes

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Abstract. The overgrowth interactions and spatial relationships amongst 7 interacting bryozoan species in the coastal waters of Bombay were studied. All interspecific encounters involved overgrowth, there being a total absence of ties. On the other hand, in intraspecific encounters, occurrence of ties was quite high. It was observed that a superior overgrowth of any species did not determine its relative abundance. None of the 7 species studied won in all its overgrowth interactions with the others. It was further observed that the success of cheilostome species to dominate over the others did not depend on their ability to grow over through any one particular encounter angle. Ranking of competitive overgrowth abilities amongst the 7 species studied did not form a simple hierarchial sequence but instead formed a competitive network.

Keywords. Epibenthic; bryozoa; competitive interactions; community structure.

1. Introduction

Any hard substratum immersed in sea water is readily colonised by a variety of solitary and colony forming marine organisms. The community patterns developed on these substrates depend on several factors such as predation, morphological features, availability and access to food, physical and chemical characteristics of the environment, composition of the biota and several other factors. The most important and limiting factor that influences the community development is the availability of space. When space for growth becomes a constraint, it is the competitive ability of the participating organisms for overgrowth that decides the community structure on hard substrates.

In recent years, a good amount of work on overgrowth competition involving an analysis of distribution and abundance of encrusting organisms (Jackson and Winston 1982) and on other related aspects such as inter-phyletic competition amongst marine benthos (Woodin and Jackson 1979), competition and cooperation interactions (Buss 1981), temporal pattern of disturbance and species diversity (Abugov 1982), life-histories and their influence on community development (Winston and Jackson 1984) and on predation and community development (Dayton 1971) have been published. Amongst these, Buss and Jackson (1979) have reported particularly on the community development of encrusting bryozoans, while the observations of Jackson (1979) refer to cryptic reef environment. Rubin (1982) who examined the interactions amongst shallow water bryozoans has also commented on the method of measurements.

The present work was carried out in the coastal waters of Bombay where the encrusting cheilostomes are perennially and abundantly present. It was felt that availability of such a material would give an opportunity to investigate the outcome of overgrowth interactions and spatial relationships amongst the 7 cheilostome species prevailing in this water. In the present study, a ranking of competitive overgrowth abilities of the 7 species was made. It was also found out if this ranking

was a simple hierarchial sequence or a competitive network (Buss 1980). Overgrowth interactions amongst cheilostomes are found to be complex and one of the factors that influences these interactions is the encounter angle formed by the interacting species. In the present study this aspect is also examined in detail.

2. Materials and methods

The test coupons made of PERSPEX, admeasuring 15×16 cm were immersed along the jetty in Bombay harbour ($18° 55'$ N lat. and $72° 50'$ E long). This environment is characterised by the presence of encrusting cheilostomes, 7 species of which are encountered here seasonally and some amongst them perennially. The site also supports the growth of a few species of ascidians, sponges, serpulids and balanids. However, these species fail to compete with cheilostomes which are recruited here in overwhelming quantity.

The test coupons, numbering over 300, withdrawn from the exposure frames after 45-50 days were air-dried and washed with fresh water to remove the detritus. The coupons were studied using a stereomicroscope for the outcome of the interspecific encounters. The encounter angle is defined as an angle between the directions of the growth of the overgrown colony and the overgrowing colony (Jackson 1979). Three encounters illustrated in figures 1 and 2 are defined as follows:

(i) *Frontal encounter.* This is an head-on encounter where the growths of two interacting colonies are in opposite directions and an encounter occurs in $121-180°$ ($181-240°$) section.

(ii) *Rear encounter.* When one colony overgrows another from behind, their growth directions are similar and the encounter occurs in $0-60°$ ($0-300°$) section.

(iii) *Flank encounter.* In this encounter, one colony overgrows along the flank of the other colony. Here the encounter occurs between $61-120°$ ($241-300°$) section.
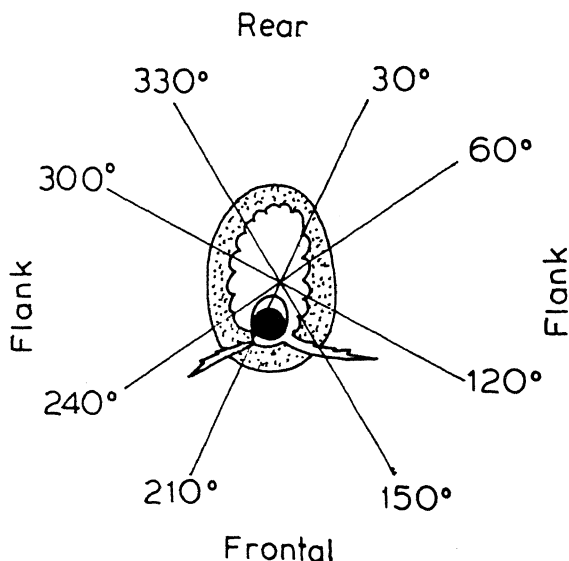
Figure 1. Categories frontal ($121-180°$ and $181-240°$), rear ($0-60°$ and $0-300°$) and flank ($61-120°$ and $241-300°$) encounters made according to the angle of encounter observed between interacting zooids in rows of colonies.
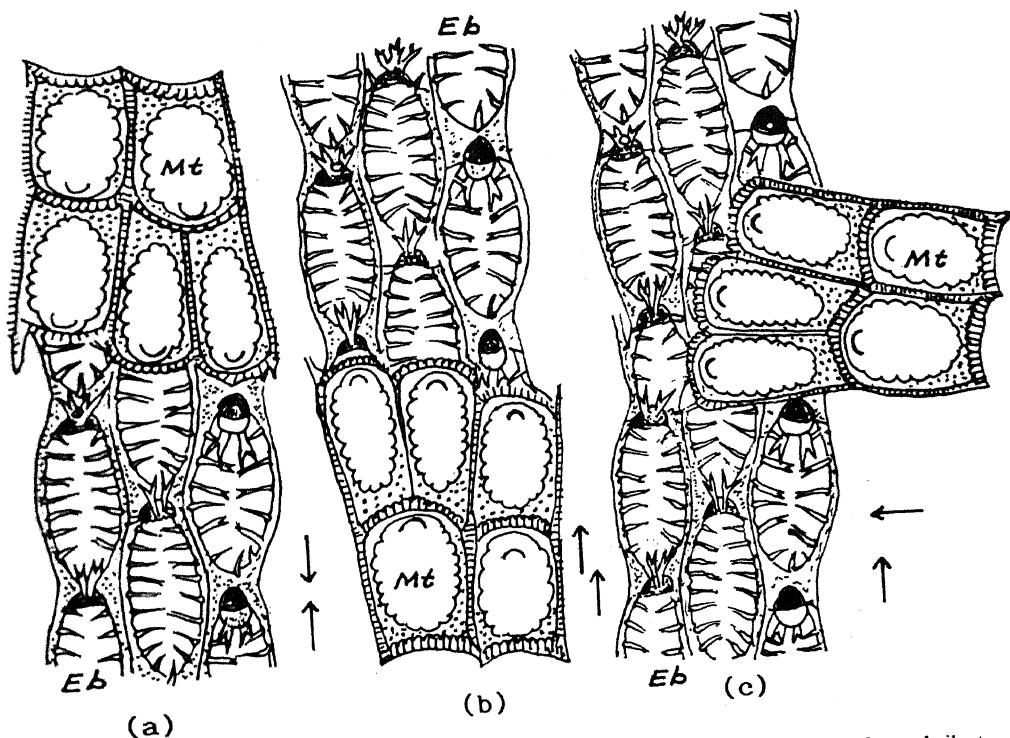
Figure 2. Categories of encounter angles between directions of growth of two cheilostomes *Membranipora tenuis* (Mt) and *Electra bengalensis* (Eb). Arrows point in direction of growth of each colony. Mt drawn as overgrowing Eb (a) frontally, (b) rearly and (c) along the flanks.

The frontal over-growth involves growing edge to growing edge encounters. The flank and rear overgrowths involve growing edge to non-growing edge encounters. Figure 3 illustrates the patterns of cheilostome encounters. The angle of encounter, that is the angle between growth directions of any two interacting colonies, was measured with the help of a microscope by rotating the ocular containing a cross-hair reticle and reading the degrees of rotation from an external scale marked at $10°$ intervals.

3. Results

3.1 *The biota*

The bryozoans cover between 50 and 70% of the area of coupons immersed at this site in Bombay harbour. Seven species to which the present data refer to are, *Electra bengalensis*, *Electra angulata*, *Membranipora tenuis*, *Membranipora annae*, *Hippoporina indica*, *Celleporaria pilaefera* and *Acanthodesia* sp.

Two species *E. bengalensis* and *Acanthodesia* sp. together cover 65·54% of the area covered by the bryozoans. The area covered by other species individually, does not exceed 6·58% of the coupon area.

3.2 *Overgrowth competition*

The number of encounters, both interspecific and intraspecific are recorded in table 1. All inter-specific encounters involve overgrowths, there being a total absence of
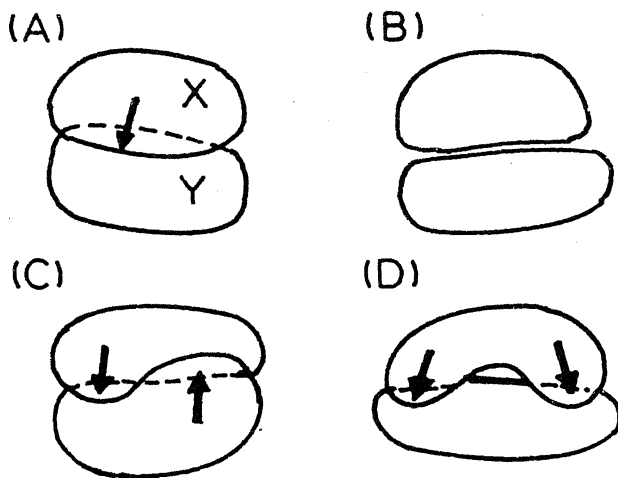
Figure 3. Patterns of cheilostome encounters. **A.** Single encounter, colony X growing over Y. **B.** Tie encounter. **C.** Colony X growing over Y and Y growing over X forming a single encounter line. **D.** Colony X growing over Y at two points showing two separate encounter lines.

Table 1. Summary of species percentage cover on test-coupons, the frequency of cheilostome-cheilostome encounters and outcome of cheilostome-cheilostome overgrowth interactions.

| Species | Number of encounters | | | | Outcome of interspecific overgrowth interactions | | | Ranking according to W/L ratio |
	Percentage cover (cm²)	Inter-specific	Intra-specific	Total	Wins	Losses	W/L ratio	
Electra bengalensis	25·9	84	50	134	35	49	0·71	6
Acanthodesia sp.	16·18	64	32	96	Nil	64	0	7
Membranipora annae	6·58	47	9	56	32	15	2·13	4
Electra angulata	4·55	44	3	47	34	10	3·4	2
Membranipora tenuis	4·11	37	10	47	25	12	2·08	5
Hippoporina indica	3·6	34	10	45	25	9	2·8	3
Celleporaria pilaefera	3·28	14	24	38	11	3	3·7	1
	64·2%	324*	138	462	162	162		

*Total encounters

ties. On the other hand, in intraspecific encounters, occurrence of ties is quite high. During this study no fusion of the colonies, both inter and intraspecific was recorded. A total of 300 cheilostome-cheilostome encounters were recorded. Of these 162 were interspecific and 138 intraspecific. This gives over 4·5 cheilostome-cheilostome encounters per 100 sq cm of the submerged surface.

Table 1 also gives the percentage area covered by individual species of these sheetlike cheilostomes and the number of interactions encountered. It is very clear from this table that more the area covered by a given species, more are the interactions, both interspecific and intraspecific. The Kendall rank order correlation of the per cent cover of each cheilostome species and the number of encounters each species involved in is $\tau = +1$ ($P < 0.0002$). This is expected of the encrusting species which grow flat and closer to the substratum.

Table 2. Pairwise (162) overgrowth interactions amongst 7 cheilostome species involved in more than 10 encounters*.

Species A	Species B						
	C. pilae-fera	E. angu-lata	H. indica	M. annae	M. tenuis	E. benga-lensis	Acantho-desia sp.
Celleporaria pilaefera		—	100 (1)	—	—	77 (13)	
Electra angulata	—		100 (1)	17 (6)	(0)	72 (14)	100 (22)
Hippoporina indica	(0)	(0)		78 (9)	34 (6)	88 (8)	100 (9)
Membranipora annae	—	83 (6)	22 (9)		50 (6)	78 (18)	100 (8)
Membranipora tenuis	—	100 (1)	66 (6)	50 (6)		53 (15)	100 (9)
Electra bengalensis	23 (13)	28 (14)	12 (8)	22 (18)	47 (15)		100 (16)
Acanthodesia sp.	0	0	0	0	0	0	

*Data are percentage overgrowth interactions in which A overgrows B (number of interactions in parentheses).

The contact matrix of 162 pairwise overgrowth interactions in which species A grows over species B are given in table 2. The number of interactions between the species pairs ranged from 0–22. These are indicated in the parentheses in the table.

Figure 4 illustrates 7 cheilostome species ranked in decreasing order of their wins/losses (W/L) ratios. This W/L ratio for any given species is defined as the number of interactions in which species A overgrows all other cheilostome species divided by number of interactions in which the other cheilostomes overgrew species A (Jackson 1979). Overall differences in the number of wins and losses for these species are highly significant (2×2 contingency table; $P < 0.0001$).

The 7 cheilostome species studied here can be divided into 3 categories on the basis of their overgrowth abilities. These are super, moderate and inferior overgrowth dominants. Three species *C. pilaefera*, *E. angulata* and *H. indica* are clearly super overgrowth dominants. Two species, *M. annae* and *M. tenuis* are moderate dominants and in the third category of inferior dominants are *E. bengalensis* and *Acanthodesia* sp. (see figure 4).

None of the 3 super growth dominants however, covers much space (3·3–4·6% of the available area) though their W/L ratios are between 2·8 and 3·7 (table 1). Moderate dominants like *M. annae* and *M. tenuis* also do not cover much space but have W/L ratios greater than 1. Two species viz *E. bengalensis* and *Acanthodesia* sp. which fall in the third category of inferior dominants, though they occupy the maximum space (together about 42%) have W/L ratios below 1. It is evident therefore that the abilities of the species to overgrow the others need not necessarily help them to occupy larger surface. On the other hand species like *E. bengalensis* and *Acanthodesia* sp., despite their handicap in overgrowing the others, succeed in occupying maximum space on the substrate.

The above observations suggest that overgrowth ability in the cheilostome-cheilostome encounters has not been an important determinant of the relative abundance of a particular cheilostome species, unlike noted by Jackson (1979) for the species assemblage he had studied in Jamaican waters. A larger the surface covered by a given species, more is the probability of its interactions with the others but not necessarily an increase in its W/L ratio.

Figure 4 will further show that none of the 7 cheilostome species wins in all its overgrowth interactions with the others. *Acanthodesia* sp. amongst these never
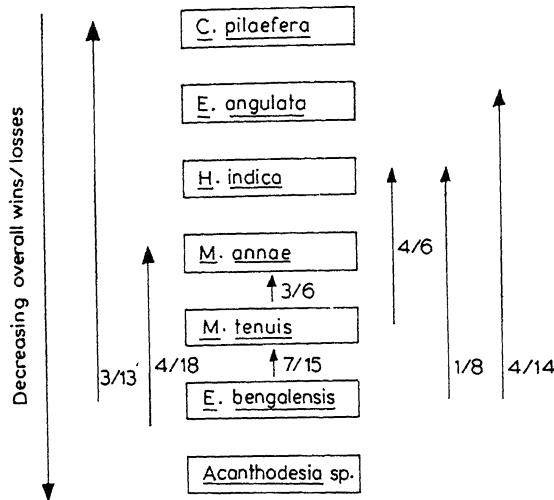
Figure 4. Ranking of 7 cheilostomes based on their W/L ratios. Upward pointing arrows indicate reversal, i.e. *E. bengalensis* out of total 13 encounters has overgrown 3 times on *C. pilaefera.*

scored a single win and was always overgrown by other species. Of the 80 pairwise overgrowth interactions between these 7 species, there were 26 reversals, i.e. cases in which the lower ranked species were observed to have grown over the species ranked above them.

It is seen from figure 4 again, that the higher ranked *C. pilaefera* is overgrown by one of the two lower ranked species, viz *E. bengalensis* during 3 of their 13 encounters. The other super-ranked species *E. angulata* is also overgrown by *E. bengalensis*. *H. indica* which is placed third in overall ranking is overgrown by two lower ranked species. *M. annae* which is ranked as moderately dominant species, is overgrown by *M. tenuis*, a species of its equal ranking and also by *E. bengalensis* that belongs to a category of inferior overgrowth dominants.

3.3 *Encounter angle and dominance*

Jackson (1979) has noted that the variations in outcome of overgrowth interactions are frequently related to (i) orientation or growth directions and associated encounter angles between colonies and their overgrowth competitions and (ii) to the conditions of the cheilostome colony surface in the region of overgrowth. In the present study, the influence of orientation factor between two competitors in their overgrowth interactions was alone examined.

The frequency of overgrowths of earlier defined 3 encounter angles for the 7 cheilostome species, (each involving in more than 10 encounters) is given in table 3. The overgrowth interaction measurements were made along the continuous encounter line between two interacting colonies. Therefore a single overgrowth may involve frontal and flank overgrowth and/or rear and flank overgrowth. Because of this, interactions recorded between two colonies, the number of rear, flank and frontal overgrowths may be more than the total number of interactions recorded.

Table 3. Outcome of interspecific overgrowth interactions at different encounter angles for 7 cheilostome species*.

Species		Percentage overgrowth interactions at each angle			Total overgrowth inter-actions
		Rear	Flank	Frontal	
Celleporaria	Won	18 (2)	9 (1)	100 (11)	11
pilaefera	Lost	0 (0)	0 (0)	100 (3)	3
	W/L	2·0	—	2·2	3·66
Electra	Won	65 (22)	56 (19)	26 (9)	34
angulata	Lost	70 (7)	50 (5)	50 (5)	10
	W/L	3·0	3·8	1·8	3·4
Hippoporina	Won	40 (10)	52 (13)	36 (9)	25
indica	Lost	33 (3)	67 (6)	33 (3)	9
	W/L	3·3	2·2	3·0	2·8
Membranipora	Won	47 (15)	84 (27)	37 (12)	32
annae	Lost	33 (5)	80 (12)	40 (6)	15
	W/L	3·0	2·3	2·0	2·13
Membranipora	Won	56 (14)	52 (13)	44 (11)	25
tenuis	Lost	91 (11)	33 (4)	42 (5)	12
	W/L	1·3	3·3	2·2	2·11
Electra	Won	91 (32)	23 (8)	51 (18)	35
bengalensis	Lost	57 (28)	57 (28)	57 (28)	49
	W/L	1·14	0·28	0·64	0·71
Acanthodesia	Won	—	—	—	—
sp.	Lost	61 (39)	40 (26)	30 (19)	64
	W/L	—	—	—	—

*Values for each angle given as percentages of total overgrowth interactions (number of interactions in parentheses). A sum of the components adds to more than 100% because any overgrowth interaction often involves more than one encounter angle.

Table 3 illustrates the percentage of encounters for each of the 7 species at the 3 encounter angles. Of the 162 interspecific, pairwise over growth interactions recorded, 44% involved frontal overgrowths, 50% flank overgrowths and 59% rear overgrowths.

Table 3 gives the outcome of interspecific overgrowth interactions at different encounter angles for the 7 species. Values given for each angle, that is rear, flank and frontal are percentage of total overgrowth interactions recorded along the 3 angles are more than the number of overgrowth interactions. For instance, for the bryozoan *E. angulata*, total interactions are 34, while total overgrowth interactions at different encounter angles are 50.

It is evident from the observations recorded in table 3 that the success of two super-dominant species viz *C. pilaefera* and *E. angulata* can not be attributed to any one particular encounter angle. In the former, W/L ratio for overgrowth interaction is higher because of frontal interactions, whereas in the latter, it is mainly due to the rear and the flank interactions. In *H. indica*, where the W/L ratio for total overgrowth interaction is 2·8, the species seems to overgrow onto others along all the 3 angles.

It is likely therefore that the success of cheilostome species to dominate over the other does not depend on its ability to grow over from any one particular encounter

angle. In other words, progressing from any one particular angle does not decide the organism's dominance. The failure of *E. bengalensis*, the prolific space occupier, in overgrowing the others, is therefore not due to its inability to overgrow through a particular encounter angle but is due to its general lack of overgrowing ability.

4. Discussion

The observations on the interactions amongst the 7 encrusting cheilostome species abundantly present in harbour waters were made. The competition for the space among these cheilostomes was very severe and one cheilostome-cheilostome encounter, either inter or intraspecific, was recorded per 22 sq cm of area. For this assemblage of 7 cheilostomes, it was observed that more the area covered by a given species, more were the interactions it encountered.

The species can be divided into 3 categories like super, moderate and inferior dominants on the basis of overgrowth abilities as decided by their win over loss ratios. The ability to dominate by any species, however, is not related to its ability to cover the space. The species *E. bengalensis* and *Acanthodesia* sp. despite their low W/L ratios occupied the maximum space. The overgrowth ability therefore did not decide the abundance of the species as noted by Jackson (1979) in the assemblage he studied. The continuous recruitment and abundance of *E. bengalensis* lead to its covering the maximum space.

The 7 species can be ranked on the basis of their competitive overgrowth abilities and it was noted that these did not form a simple hierarchial sequence but instead formed a competitive network. None of the 7 cheilostome species won in all its overgrowth interactions with the others. The higher ranked species like *C. pilaefera* was overgrown by a lower ranked species *E. bengalensis*. Outcome of the overgrowth interactions between colonies of the same two species was not the same nor was the outcome between two species of the same genus. These observations therefore, support the view of Osman (1977) that 'it is extremely unlikely that the outcome of any two species interaction involving sessile colonial animals will always be the same'. As stated by Jackson (1979) 'no species is likely to win all of its competitive interactions and rankings of species, competitive abilities will rarely, if ever, form a simple hierarchial sequences'.

Jackson (1979) has noted that interactions between encrusting cheilostomes are complex and that variations in outcome are significantly correlated with the encounter angle formed by the intersection of the growth direction/vectors of interacting colonies. The present study involving an assemblage of 7 species shows that the success of any cheilostome species to dominate over the others does not depend on its ability to grow over from any one particular encounter angle.

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Seasonal changes in the level and content of different biochemical constituents in tropical cerithiids *Cerithidea* (*Cerithideopsilla*) *cingulata* (Gmelin 1790) and *Cerithium coralium* Kiener 1841

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Abstract. The level and content of different biochemical constituents were investigated over a period of one year (January–December 1982) in foot, gonad digestive gland complex and viscera of two tropical cerithiids, inhabiting two different regions of backwaters. Their seasonal indices exhibited bimodal pattern of cycling and this trend was more predominant in gonad digestive gland complex and foot. In all the body components of both species, the biochemical content was found to show considerable changes when compared to their levels. In both the cerithiids, the gonad digestive gland depicted remarkable variations with the season, followed by foot. The viscera did not show much variation when compared to other body components. The carbohydrates and glycogen of both the species showed a primary peak in March and a secondary peak in September indicating maturity of gonads. This was followed by a decline in their constituents which might be due to spawning. Lipid utilisation was also observed in both the animals next to carbohydrates and glycogen. Proteins were least utilised in the process of reproduction. Thus a carbohydrate-oriented metabolism was recorded in both the species. The results were compared with the other molluscs.

Keywords. Biochemical level; biochemical content; *Cerithidea cingulata*; *Cerithium coralium*; biochemical constituents; carbohydrate-oriented metabolism.

1. Introduction

The two vital processes, reproduction and growth, of many invertebrates require energy which may be stored in reproductive or somatic body parts (Giese 1969). The existing literature (Giese 1959, 1966, 1969; Stickle 1975; Nagabhushanam and Mane 1975; Belisle and Stickle 1978; Deshpande and Nagabhushanam 1983) reveals a close relation between reproduction and changes in biochemical constituents stressing the importance to study the biochemical contents to arrive at a definite conclusion. Among molluscs, bivalves (Bayne 1976) have received much attention. Stickle (1975) and, Belisle and Stickle (1978) have made investigations on this aspect in the inter-tidal gastropods *Thais lamellosa* and *Thais haemastoma* respectively. Recently Uma Devi *et al* (1985) reported the changes in the composition of different biochemical constituents in an intertidal gastropod *Morula granulata*. Chitons have also been used for this study by Lawrence and Giese (1969), Himmelan (1978) and Deshpande and Nagabhushanam (1983). But no information was available on this topic in cerithiids. Therefore, in the present investigation, an attempt has been made to study the seasonal changes in the biochemical constituents of *Cerithidea cingulata* and *Cerithium coralium*. Both the level and content of different biochemical constituents have been studied and a comparison has also been made between the animals. It is also interesting that there is a difference in their distribution: *C. cingulata* lives in the middle reaches of the estuary whereas *C. coralium* occurs in the lower reaches of the estuary.

2. Materials and methods

Medium sized *C. cingulata* (shell length 27–31 mm) and *C. coralium* (shell length 23–28 mm) were collected from the backwaters of Bhimilipatnam which is 25 km from Visakhapatnam on the east coast of India. They were equilibrated to the laboratory conditions for about 24 h during which period the gut contents were cleared. Ten animals of each species were dissected and the different body components were separated. The same components of all the animals were pooled separately for further analysis. The body components of each species consist of foot, gonad digestive gland complex and viscera. As the gonad and digestive gland are inseparable, they are taken together as gonad digestive gland complex. Their wet weights were taken and dried in an air oven at 60°C. The dry tissue powder of each body component was prepared and it was stored in glass vials kept in desiccator. Collections were made for every month over a period of one year (January–December 1982). The different body component indices were calculated by using the method of Stickle (1975).

The dry tissue powder of each body component was analysed for different biochemical constituents viz., carbohydrates, glycogen, proteins and lipids. Anthrone method (Carrol *et al* 1956) was used for the estimation of carbohydrates and glycogen. Protein estimation was made by using Folin phenol reagent (Lowry *et al* 1951). The determination of lipids was carried out with chloroform:methanol (2:1) as suggested by Folch *et al* (1959). The total ninhydrin positive substances (TNPS) were estimated by the method of Moore and Stein (1954).

The different biochemical constituents are presented as percentage of dry weights. Biochemical content (total present per component of a 100 g standard animal) was calculated by using the method of Stickle (1975). ANOVA (Snedecor and Cochran 1967) was carried out to determine the statistical variation in different biochemical constituents. Further analysis of the data was done by Duncan's multiple range test (Snedecor and Cochran 1967) to determine whether the variations are significant.

Results

3.1 *Field observations*

The animals, *C. cingulata* and *C. coralium*, inhabit two different regions of the backwater system where the environmental conditions fluctuate with the season (Prabhakara Rao 1980). This backwater system is extensively shallow covering about 4·5 sq. km adjoining the coast. A small river, Gousthani, and 3 freshwater creeks empty into this backwater system. The backwater system is connected to the bay waters through a narrow entrance channel.

C. cingulata is found in the middle region of the backwater system. The salinity regime at this region oscillates between 6·81 and 23·22‰. There is considerable influx of sewage and land drainage. The dissolved oxygen levels vary from 10·22–2·04 ml O_2^{-1}. The temperature fluctuations in this region are: 28·5–34·8°C. The movement of water is very slow and practically this is void of wave action and presents a stagnant brackish condition. The organic matter is plentiful in this region. The substratum is composed of medium sized grains of sand (0·350–0·250 mm).

C. coralium lives near the lower region of the backwater. Here the conditions are almost marine with frequent disturbances. The salinity of the water varies between 20·23 and 34·02‰. *C. coralium* occurs in marine conditions and was never found to extend into the upper reaches of backwaters. The temperature in this region ranges from 25·5–34·8°C. The dissolved oxygen levels vary from 5·656–3·7 ml O_2^{-1}. The organic matter is comparatively lower in this region than in the habitat of *C. cingulata*. Coarse type sand grains (0·710–0·500 mm) are present in this region.

There are two spawning periods in these cerithiids: one occurring in June and July and another in December and January. During these periods, the animals were found to lay eggs in the form of ribbon on any substratum available for them.

3.2 Body component indices

All the different body component indices were found to be higher in *C. cingulata* than *C. coralium* (figure 1). However, both species exhibited a bimodal seasonal pattern in their indices with primary and secondary peaks. In foot, a primary peak value was observed in March and a secondary peak in September for *C. cingulata*. The highest
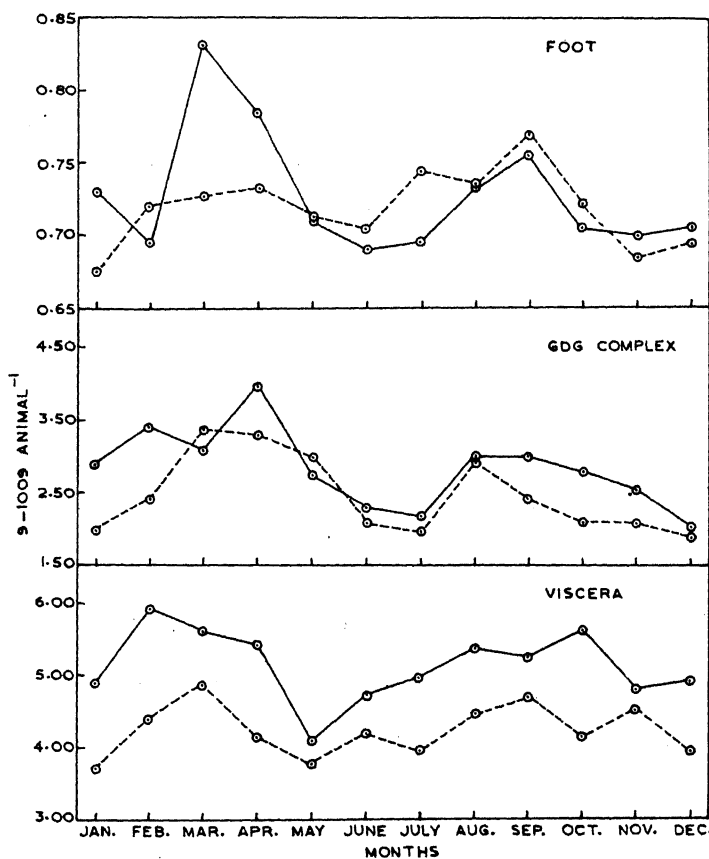
Figure 1. Seasonal changes in the body component indices of *C. cingulata* (———) and *C. coralium* (– – – –).

foot value was recorded in *C. coralium* in September. The gonad digestive gland complex index of *C. cingulata* was highest in April and a secondary peak was noticed in August. In *C. coralium*, the gonad digestive gland complex exhibited a peak value in March and another peak in August. The viscera of both species did not show any particular trend but highest value was observed in February for *C. cingulata* and in March for *C. coralium*.

3.3 *Seasonal changes in biochemical composition*

The changes in the levels of different biochemical constituents in *C. cingulata* and *C. coralium* are presented in figure 2 for foot, figure 3 for gonad digestive gland complex and figure 4 for viscera. It is clear from ANOVA that the changes (both
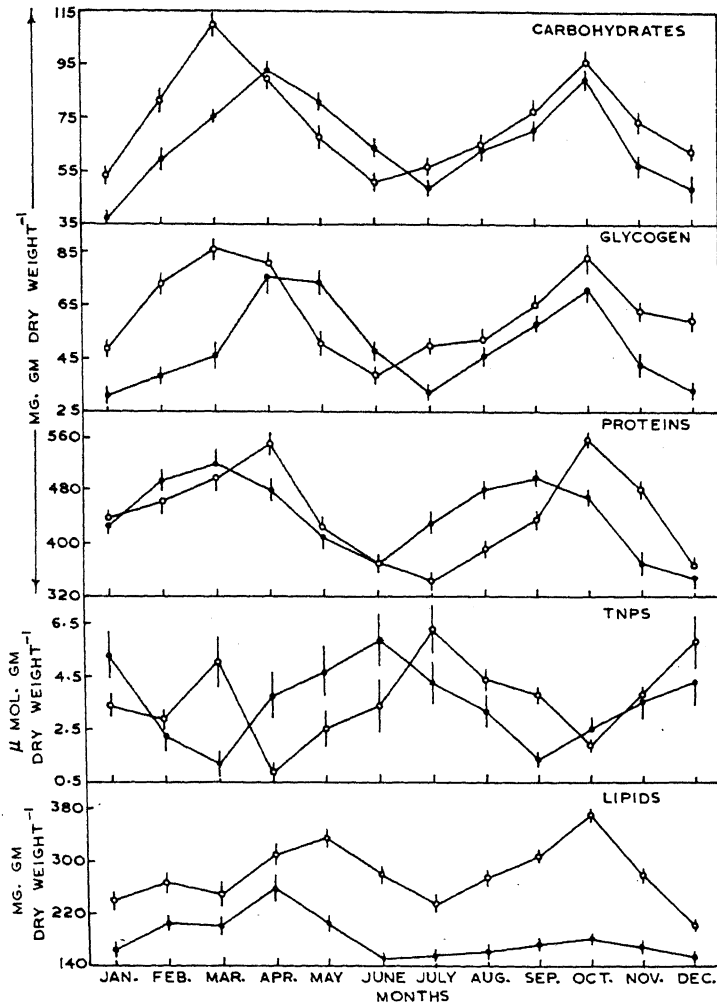
Figure 2. Seasonal changes in the levels of different biochemical constituents in foot of *C. cingulata* (○) and *C. coralium* (●).
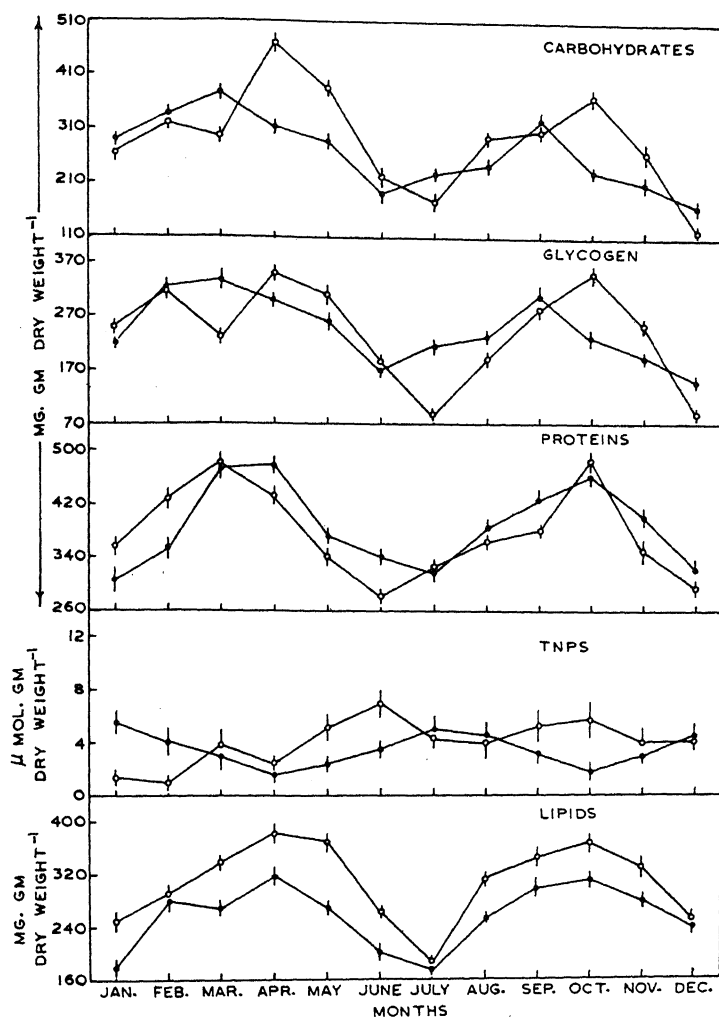
Figure 3. Seasonal changes in the levels of different biochemical constituents in gonad digestive gland complex of *C. cingulata* (○) and *C. coralium* (●).

level and content) in biochemical constituents for different body components i.e. foot, gonad digestive gland complex and viscera were found to be significantly different ($P < 0.05$) for both the species.

4. Discussion

The present investigation demonstrates seasonal changes in different body component indices and also in the biochemical constituents (level and content) of both species. The bimodal pattern of indices observed in the present investigation correlates well with spawning period of the animals. During the months of February and March, the increase in the index values can be attributed to the addition of new cellular elements in the form of gonad maturation. The secondary maximum appeared
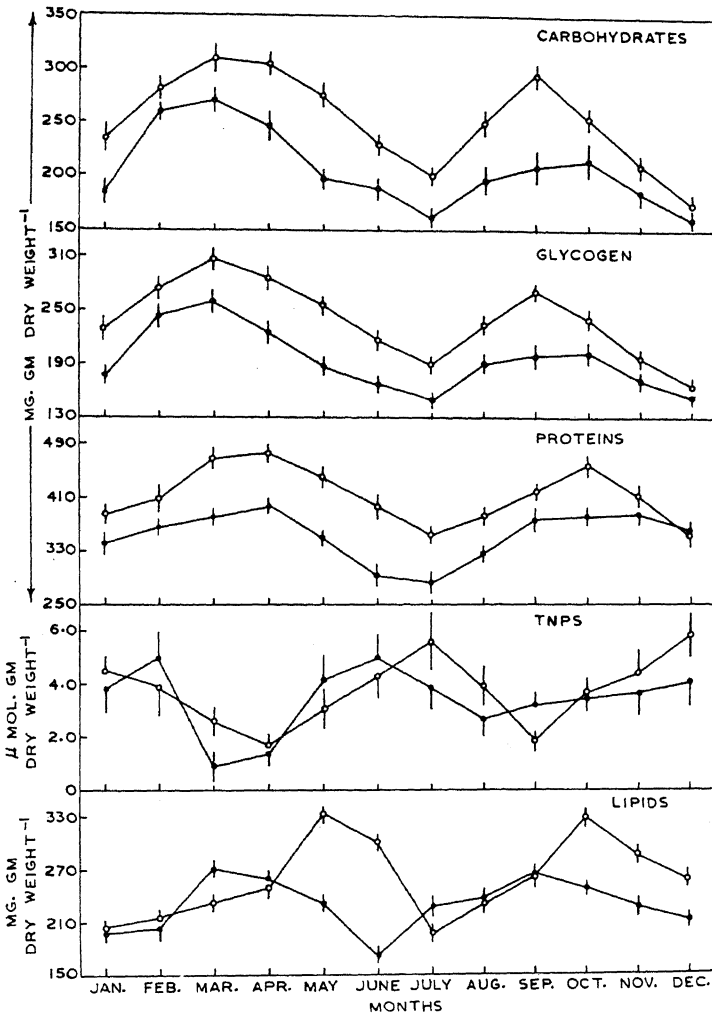
Figure 4. Seasonal changes in the levels of different biochemical constituents in viscera of *C. cingulata* (○) and *C. coralium* (●).

in the months of September and October which may denote the preparation period for the minor spawning season. Similar increase in gonad index during reproductive season was reported in several molluscs (Giese and Pearse 1974). Lawrence *et al* (1965) have noticed this type of cycling of gut and gonad indices in *Katherina tunicata* and *Cryptochiton stellari* respectively during reproductive phase. A rapid increase in the gonad index value was due to accumulation of ripe gamates which are to be released during spawning season (Giese 1969). The low values observed in July and December for both the species indicate the period of spawning. Nagabhushanam and Deshpande (1982) also reported seasonal variation of testis, gonad and digestive gland indices in *Chiton iatricus*. No cyclic pattern was noticed in the digestive gland of abalone *Haliotis cracheroidii* (Webber 1970). However, the higher indices observed in *C. cingulata* than *C. coralium* might be due to greater accumulation of gamates or to a thinner shell in *C. cingulata*.

A close observation of the results indicate that of all the 3 body components, major changes were observed in gonad digestive gland complex and to some extent in foot. According to Giese (1969) that there are no discrete biochemical storage organs in molluscs as occurred in vertebrates. Therefore, during the period of reproduction which requires a great amount of energy, gonad digestive gland complex contributes much and then followed by foot. In foot also, reserve food material may be stored for use but only a small amount of utilisable biochemical constituents are channeled out during reproductive period. It is clear from the results that the carbohydrates and glycogen played a major role during reproduction when compared to other biochemical constituents in both species. The changes observed in biochemical levels indicate their synthesis and utilisation during different periods, but their contents depict a clear picture of storage and utilisation (Stickle 1975). The content values observed here, thus, denote remarkable changes showing their accumulation and utilisation.

The biochemical constituents, mostly carbohydrates and glycogen, were high during February–April indicating the maturation of gonads followed by spawning in May–July in which months low values were recorded. These constituents again increase during prespawning period (August and September) and there is second maxima in October. Then, during November–January, there appeared a second spawning season with low values of biochemical constituents indicating the shed down of male and female gametes. Thus, a bimodal pattern of reproductive cycle with accumulation and utilisation of different biochemical constituents, mostly carbohydrates and glycogen, was observed in both species. A similar bimodal pattern was reported in gastropod *Littorina irrorata* but with peaks in May and August (Bistransin 1976). However, a unimodal pattern is not uncommon in cold water gastropods as observed by Stickle (1973) in *Thais lamellosa* in which the TDG, ODG and CAG were found to cycle with the season. Belisle and Stickle (1978) studied seasonal changes both in level and content of carbohydrates, protein and lipid in different body components of *T. haemastoma* and they found a bimodal pattern of seasonal change. They have also reported that the changes in different biochemical constituents were predominant due to component indices but not to fluctuations in constituent levels. Bistransin (1975) observed a bimodal pattern of seasonal changes in the RVM of *L. irrorata* with peaks in May and August. In *Clypeomorus clypeomorus*, an intertidal cerithiid, high amounts of biochemical constituents were observed from June–August and December–February due to development of gonads (Manmadha Rao 1977). The increase in different biochemical constituents viz. glycogen, protein and lipid during gonad development was reported in several molluscs, *Parreysia corrugata* (Nagabhushanam and Lomte 1971), *Paphia laterisulca* (Nagabhushanam and Dhamne 1977), *Mytilus viridis* (Nagabhushanam and Mane 1978), *Villorita cyprinoides* var. *Cochenensis* and *Meretrix casta* (Lakshmanan and Krishnan Nambison 1980). Deshpande and Nagabhushanam (1983) while studying the seasonal changes in the biochemical composition of *C. iatricus* and *K. tunicata*, suggested an increase of the glycogen content with the ripening of testes and ovaries in both the species indicating its synthesis and utilisation during reproductive period. Recently, Uma Devi *et al* (1985) reported a progressive increase of all biochemical constituents during May–October and a decrease during November–April in *M. granulata* and they have correlated these changes to reproductive season and feeding of the animal.

Food availability is also one of the factors that influence the biochemical

constituents of the animals (Ansell 1974). In the present investigation, the food availability in the habitat of *C. cingulata* is more when compared to *C. coralium*. Both of them feed on algae (*Ulva* and *Enteromorpha* sps.) and myriads of benthic diatoms. Because of frequent disturbances, the algal growth is found to be not much in the lower reaches. This may be one of the probable reasons for the presence of greater amounts of biochemical constituents in *C. cingulata* than *C. coralium*. However, the run-off from the river may also bring nutrients for *C. cingulata*. It was also reported (Annapurna 1978) that organic composition was found to be higher in the habitat of *C. cingulata*. As the environmental conditions in the habitat of *C. cingulata* show wide variations, the animal has to withstand all these problems and reproduce. Therefore, they need more energy for propagation and the reproductive material should also be produced much to counter the environmental disturbances. Hence, these animals of *C. cingulata* have stored more quantities of biochemical constituents than *C. coralium*.

It is interesting to observe a carbohydrate-oriented metabolism in these cerithiids as the glycogen and carbohydrates are the major biochemical constituents utilised during reproduction. These cerithiids also exhibited a carbohydrate-oriented metabolism when they were subjected to starvation stress (Prabhakara Rao *et al* 1987) and oxygen lack (Prabhakara Rao and Prasada Rao 1983). Thus, whenever, these animals are in need of energy, they switch on to carbohydrate reserves. Similarly carbohydrate-oriented metabolism was reported in several molluscs (Emerson 1967; Ramamurti and Subrahmanyam 1976; Manmadha Rao 1977; Nagabhushanam and Mane 1978). Next to carbohydrates, these animals preferred lipids and lastly proteins are utilised. According to Giese (1959), only 5% of the total lipids constitute for the structural lipids and the rest might be a storage for utilisation. Moreover, Lawrence and Giese (1969) also reported increased utilisation of lipids during gamatogenesis. However, protein content was not much utilised in cerithiids but in some of the other molluscs, proteins appeared to be the major biochemical reserves for utilisation during reproduction (Giese and Araki 1962; Stickle 1975; Deshpande and Nagabhushanam 1983). The TNPS values of both the animals showed decline in March and April because of utilisation for the formation of proteins. Another set of minimum values occurred in September and October. The highest values in June and July and, December and January might be due to proteolysis and they are in synchronisation with protein depletion.

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Effect of feeding organic acid treated maize (*Zea mays* L.) on growth and reproduction of albino rats (*Rattus norvegicus albinicus*) and Indian desert gerbils (*Meriones hurrianae*, jerdon)

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Abstract. The organic, acetic and sorbic acids at 1% level were fed to albino rats and Indian desert gerbils in whole and broken maize corn. The gerbils were highly sensitive to maize treated with organic acids. The reproduction studies showed that the gerbils had more number of abnormalities with regard to the young ones. The sorbic acid treated maize possessed a teratogenic potential and it induced cannibalism. In case of albino rats no such abnormalities were found. However, abortion, delayed gestation period and cannibalism were common. Based on the data obtained it was postulated that the gerbils could be used as alternatives to albino rats in toxicological studies.

Keywords. Organic acids; maize; reproduction; albino rats; desert gerbils.

1. Introduction

The antimicrobial food additives such as sorbic acid and acetic acid are known to have an antimicrobial control potential (National Academy of Sciences 1973). The use and official regulation of acid treated grains in food and feed products is limited partly by lack of data on behaviours of the acids in food systems and effect on animal nutrition. Sorbic and acetic acids have been approved by the food and drug administration (FDA) as generally recognised as safe (GRAS). While, no report exists on the chronic toxicity of acetic acid or acetates when used as food preservatives (Lueck 1980) much work has been carried out with sorbic acid. Sorbic acid is known to possess low mammalian toxicity (Gaunt *et al* 1975) properties with ovicidal action (Dunkel *et al* 1982; Dunkel and Read 1986). Hence, it has been approved by the environmental protection agency (EPA) for use on whole grain and other raw agricultural commodities (Federal Register 1982). Hence, the present study was undertaken to study the effect of acetic and sorbic acids on the growth and reproduction of albino rats and Indian desert gerbils.

2. Materials and methods

2.1 *Animals and diet*

Fifteen adult female and male albino rats (*Rattus norvegicus albinicus*, Wistar-CFT strain) and Indian desert gerbils (*Meriones hurrianae*, jerdon) bred and reared in the CFTRI animal house, weighing between 180–250 g were selected and grouped randomly.

2.2 *Preparation of experimental diet*

Freshly harvested maize grain (50 kg) was determined for its moisture content and the presence of aflatoxins B_1, B_2, G_1 and G_2 (Pons *et al* 1966). Maize was ground in a laboratory mill and 1% sorbic acid (Sorbinosoüre, E Merck, Germany) was incorporated (10 g/kg) into the maize flour and sieved through a wire mesh (20 mesh size) for even mixing. Similarly 1% acetic acid (10 ml/kg) was added to the grain mixed thoroughly for even coating and then passed through the mill. This flour containing the organic acids were fed to the albino rats.

The 1% dose of acids was selected from the pilot studies during which 0·05–3% acid doses were used. Dose of both the acids (1%) controlled all the mycoflora (mostly storage fungi) and also significantly suppressed the F_1 progeny of the test insects (Ameeta Banerjee 1986). For the gerbils, the grain was treated with the organic acids as mentioned above except that the grain was broken into small pieces in the mill and then fed. The control diets were maize without any acid treatment.

2.3 *Embryotoxic studies in albino rats and Indian desert gerbils*

2.3a *Experimental design*:   Fifteen pairs of albino rats and gerbils (1 : 1 ratio) were caged in pairs for mating and divided into 3 groups. The first group received the control diet. The second group was fed with maize diets treated with 1% acetic acid, while the third group received maize diets treated with 1% sorbic acid. The diets were fed *ad libitum* for a period of 60 days and the daily food consumption was monitored by weighing the residues. Drinking water was provided all the 24 h. Adult animals were studied for body weight gain and haematological parameters. After day one of pregnancy the males were separated and the females were allowed to litter and rear their young ones upto weaning. The offsprings were observed for survival, physical and functional development. At the end of 60 days, the animals were autopsied using a diethyl ether anaesthesia. Liver, kidney, ovary and testis were excised, blotted to remove the adhering blood and weighed on a mettler balance nearest to mg and the organ weights were determined.

2.5 *Measurement of haematological parameters*

Blood was drawn by cardiac puncture and aliquot was taken in tubes containing EDTA for haemoglobin (Hb) and white blood counts (WBC). WBC's were estimated by a haemocytometer (Bharucha *et al* 1976). Differential count of WBC was made by wright's staining technique. Hb was determined by the cyanmethamoglobin method using the commercial kit (Bharat Laboratories, Thane).

3. **Results and Discussion**

The moisture content of the grain was found to be 13·5% and the maize sample was found to be negative for any of the aflatoxins tested. There was no consistent differences between the treated and control albino rats and gerbils in food consumption. The rate of body weight gain of both the species of rats given 1% sorbic acid

and 1% acetic acid did not differ significantly from that of the controls. However, observations of a slight increase or decrease in the body weight of the animals could be due to the food intake pattern as there were inconsistencies in food intake. The increase in the body weight with sorbic acid has been observed by other workers (Deuel *et al* 1954; Gaunt *et al* 1975).

There were no significant alterations in the relative organ weight of the animals (table 1). The only organ showing an increase in terms of absolute weight was the ovaries in female gerbils and albino rats fed 1% sorbic acid. A statistically significant decrease ($P < 0.05$) in the testis of the male albino rats fed 1% acetic acid was also evident. The slight increase in the weight of the liver observed could be interpreted as a functional hypertrophy.

The findings of the haematological parameters are shown in table 2. As evident from the table, there was a statistically significant decrease ($P < 0.05$) in the WBC count accompanied by a statistically significant decrease ($P < 0.01$) in the neutrophils of the male albino rats fed with 1% sorbic acid. A statistically significant increase ($P < 0.01$) in the differential count of the neutrophils was also evident in the female albino rats fed with 1% acetic acid. Though, a decrease in the white blood cells accompanied by the decrease in lymphocytes of the Indian desert gerbils fed with 1% acetic acid and sorbic acid treated maize was also evident, the values obtained were not statistically significant from those of the controls.

The above changes suggest the possibility of the chemicals especially the acetic acid and sorbic acid to disrupt the immunological responses of the gerbils as reflected by the decreased count of the lymphocytes. Roitt (1977) reported such changes in lymphocytes in albino rats with certain pesticides. The increased neutrophil count might be related to the response of infectious agents or xenobiotics in the host environment as postulated by Wintrobe (1976). However, the exact mechanism by which the changes in the haematological parameters were evoked could not be known.

The data on reproduction studies in albino rats and gerbils are presented in table 3. The reproduction performance of the rats was affected as evident from the abnormalities found in the young ones. In the sorbic acid treated group two out of five female albino rats gave birth to one pup each and the rest of the foetus aborted. The animals on autopsy showed resorption of the embryo which were still formed. In the gerbils too the same effect was observed. It was noted that two out of the five female gerbils gave birth to malformed pups. The malformations were of the fore, the hind limbs and the tail. In another the female gerbil gave birth to a pup which survived for 14 days and later died. X-ray scanning of the 14 day old pup revealed gross growth retardation, microcephaly with spina bifida and teratoma in the abdomen. Sorbic acid also induced cannibalism. With 1% acetic acid treated maize no such abnormalities were found in the rats but delayed gestation period, resorption of the embryos and abortion were observed. Demaree *et al* (1955) reported that 10% sorbic acid in the diets of albino rats had no effect on the reproduction. While, O'Dell *et al* (1948) demonstrated that a deficiency of folic acid was the chief cause of congenital abnormalities. Later O'Dell *et al* (1951) found that a high proportion of the offsprings are afflicted with abnormalities if the mothers are severely depleted of vitamin B_{12}. The present study indicated that sorbic acid treated maize could induce congenital abnormalities. As the mode of action of sorbic acid and acetic acid is not known, the abnormalities found could not be directly related to the organic acids nor

Table 1. Organ weight of rats fed with maize diets containing 1% acetic acid and 1% sorbic acid for 60 days.

Animals (rats)	Organs	Male			Female		
		C	AA	SA	C	AA	SA
Meriones hurrianae (Jerdon)	Terminal body weight	101·75 ± 7·31	107·25 ± 13·41	108·00 ± 8·89	96·6 ± 3·45	96·00 ± 6·37	94·5 ± 7·57
	Liver	2·97 ± 0·10	3·47 ± 0·75	2·846 ± 0·30	3·12 ± 0·14	3·11 ± 0·26	3·03 ± 0·14
	Kidney	0·55 ± 0·026	0·52 ± 0·05	0·45 ± 0·05	0·94 ± 0·28	0·52 ± 0·02	0·58 ± 0·04
	Testis/ovary	0·630 ± 0·033	0·525 ± 0·04	0·607 ± 0·06	0·027 ± 0·008	0·027 ± 0·006	0·073[a] ± 0·009
Rattus norvegicus albinicus	Terminal body weight	236·33 ± 11·33	245·66 ± 2·02	228·66 ± 10·7	238·00 ± 27·38	231·00 ± 5·567	241·00 ± 13·79
	Liver	3·433 ± 0·19	3·278 ± 0·02	3·712 ± 0·12	3·276 ± 0·37	3·204 ± 0·09	3·531 ± 0·29
	Kidney	0·572 ± 0·02	0·508[a] ± 0·01	0·562 ± 0·01	0·489 ± 0·03	0·484 ± 0·03	0·472 ± 0·02
	Testis/ovary	1·245 ± 0·03	0·946[a] ± 0·10	1·233 ± 0·07	0·045 ± 0·06	0·041 ± 0·003	0·058[a] ± 0·05

Values are mean ± SE of 4 animals; Students 't' test [a] $P < 0.05$.
C, Control; AA, acetic acid; SA, sorbic acid.

Table 2. Blood picture of rats fed with maize diets containing 1% acetic acid and 1% sorbic acid for 60 days.

Animals (rats)	Blood parameters	Male			Female		
		C	AA	SA	C	AA	SA
Rattus norvegicus albinicus (Albino rats)							
	Hb (g/dl)	13·16±1·09	13·5±0·06	12·0±1·04	14·0±0·8	13·0±0·86	13·16±0·44
	WBC (µl)	14,550±1,841	10,750±1,970	8,950^a±781	11,433±1,186	10,100±1,985	13,500±1,719
	Differential count (%)						
	Lymphocytes	67·33±2·40	71·0±3·51	61·66±23·66	76·66±6·38	65·33±7·26	78·0±1·15
	Neutrophils	32·33±2·33	28·33±3·38	13·66^b±1·76	23·0±6·08	33·66^b±7·62	21·66±0·88
	Monocytes	—	0·66±0·33	—	0·33±0·3	1·0±0·57	0·33±0·3
Meriones hurrianae (Jerdon) (Desert gerbil)							
	Hb (g/dl)	13·25±0·43	14·12±0·65	14·25±0·59	13·25±0·25	13·37±0·55	12·87±0·12
	WBC (µl)	9,450±2,810	6,362±528	5,865±5,489	9,500±1,934	5,775±1,214	9,300±3,403
	Differential count (%)						
	Lymphocytes	74·5±2·62	71·25±6·56	68·5±1·70	82·25±1·75	81·25±4·95	68·75±8·48
	Neutrophils	24·25±2·78	28·75±6·56	30·5±1·5	16·75±1·54	18·0±5·04	30·75±8·66
	Monocytes	1·25±0·47	—	1·0±0·57	1·00±0·44	0·15±0·75	0·5±0·5

Values are Mean±SE of 4 animals each; Students 't' test ^{a}P<0·05; ^{b}P<0·01.
C, Control; AA, acetic acid; SA, sorbic acid; Hb, Haemoglobin; WBC, white blood cells.

Table 3. Results of reproduction study in rats fed maize diets containing 1% acetic acid and 1% sorbic acid for 60 days.

Animals (rats)	Dietary level (%)	No. of females pregnant/ No. mated	No. of litters born	Mean pup wt (g) at day	No. of dead pups	No. of live pups	No. of resorp-tions/ pregnant female	No. of sterile males
Meriones hurrianae	Control	5/5	12	3·0	0	12	Nil	0/5
(Jerdon)	1% Acetic acid	3/5	9	2·38	0	8	5	2/5
	1% Sorbic acid	5/5	6	2·7	8	Nil	8	0/5
Rattus norvegicus	Control	5/5	35	4·37	2	33	Nil	0/5
albinicus	1% Acetic acid	4/5	15	3·73	0	15	23	1/5
	1% Sorbic acid	4/5	12	2·87	12	0	10	1/5

to the mycotoxins as the sample was found to be free from it. However, it can be stated that the sorbic acid treated maize possessed a teratogenic potential.

It is therefore, essential that further studies be carried out for evaluating the safety of these much used antimicrobial food additives. It should be noted that there are very few published reports on the effect of sorbic acid on reproduction (Demaree *et al* 1955) which suggested that sorbic acid at 10% level had no effect on reproduction. The present findings are in disagreement with the earlier reports of Demaree *et al* (1955). The present study revealed that sorbic acid in combination with some low protein and vitamin diet like maize exerts an adverse effect on reproduction of gerbils and albino rats. The teratogenic potential of the sorbic acid treated maize could be due to the above mentioned fact. Such studies on gerbils seem to have not been carried out earlier.

The response with respect to embryo toxicity under the influence of dietary organic acids like the sorbic and acetic acids seem to have opened a new field of future research for using Indian desert gerbils as alternatives to albino rats as laboratory animals.

Acknowledgements

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Fecundity of the Chinese silver carp *Hypophthalmichthys molitrix* (Val.) from Gujartal Fish Farm, Jaunpur, UP

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Abstract. Data on the fecundity of *Hypophthalmichthys molitrix* was statistically computed and the relationships between fecundity and body measurements were found to be linear. The fecundity was more closely related to the body weight ($r = 0.8439$, $F = 15.37$ at 1% level of significance) than fish length ($r = 0.704$, $F = 8.953$ at 5% level of significance) and the ovary weight ($r = 0.6794$, $F = 8.3839$ at 5% level of significance).

Keywords. Fecundity; Chinese silver carp.

1. Introduction

The knowledge of fecundity and its relationship with the body measurements make it possible to estimate the number of eggs that are likely to be spawned by the fish. In fish culture, if the number of eggs likely to be obtained from the spawning stock is known, it is easier to make arrangement for their successful hatching. The silver carp, *Hypophthalmichthys molitrix* (Valenciennes) naturally occurs in the river system Yangtze, West river, Kwangsi, Kwangtung in south and central China and in the Amur basin in USSR. In India, the first ever consignment of 360 fingerlings of silver carp was brought from Japan in 1959 to the pond culture division of Central Inland Fisheries Research Institute, Cuttak, Orissa. In due course, they were bred successfully by hypophysation and their fry were distributed from Cuttack to various states of India (Jhingran 1982).

H. molitrix is a planktivorous or surface feeder fish which breeds during April–July in its natural habitat in China and in the month of June–July in the Tone river, Japan. At Gujartal Fish Farm this species is an important member of the composite fish culture. The present study will be of greater help in making the proper management for the successful hatching of eggs.

2. Materials and methods

Mature specimens of *H. molitrix* were selected and all the body measurements were recorded in fresh condition. The ovary of each fish was dissected and preserved in 5% formalin solution. The fecundity of fish was recorded by gravimetric count method and studied in relation to total length, body weight and the ovary weight of fish. For the total fecundity estimation, 3 random samples of 100 mg each were taken from the anterior, middle and posterior regions of each ovary of every specimen. The number of ova in each sample were computed under a binocular microscope and total number of eggs in each ovary were estimated by the following formula:

$$F = S \times OW/100,$$

where F is fecundity, S is average number of eggs obtained from 6 different samp
of 100 mg each and OW is total weight of ovary. The relationship between fecund
and other body parameters were obtained by plotting the respective values as
scatter diagram and fitting the straight line equation:

$$Y = a + bX,$$

where Y is independent variable, i.e. fecundity; X is dependent variable, i.e. f
length, fish weight and ovary weight, a and b are the constants. The correlati
coefficient, r, was calculated for each relationship. The analysis of variance (F) w
made use for testing the linearity of regression.

3. Results

3.1 *Fecundity and fish length*

The relationship between fecundity and fish length is shown in figure 1. The num
of ova varied from 1·14 lakh for a fish of 43·6 cm to 2·55 lakh in the fish measur
49·5 cm. The largest specimen of 50·4 cm had a fecundity of 2·49 lakh. The relati
ship between fecundity and the total length was observed to be as:

$$F = -8·1946 + 0·21339 \, L \qquad (r = 0·70407),$$

where F is fecundity, L is fish length and r is correlation coefficient. The analysis
variance proved the linearity of regression (observed $F = 8·9532$, significant at
level).

3.2 *Fecundity and fish weight*

The relationship between fecundity and the fish weight is expressed in figure 2. F
production ranged from 1·14 lakh in a fish of 0·760 kg to 2·55 lakh in a fish
1·350 kg. The mean values of the fecundity calculated per kg body weight ran
from 1·483 to 1·78 lakh. The fecundity-body weight relationship can be expressed

$$F = -0·6529 + 2·1884 \, FW \qquad (r = 0·843904),$$
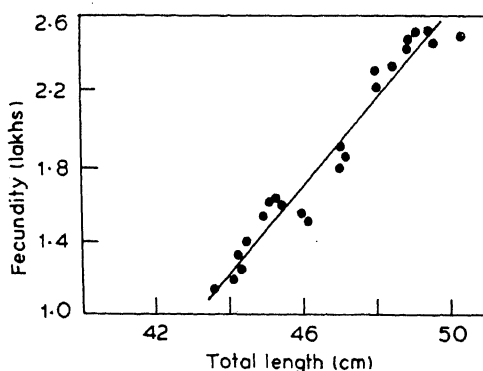
Figure 1. Relationship between fecundity and total length in *H. molitrix*
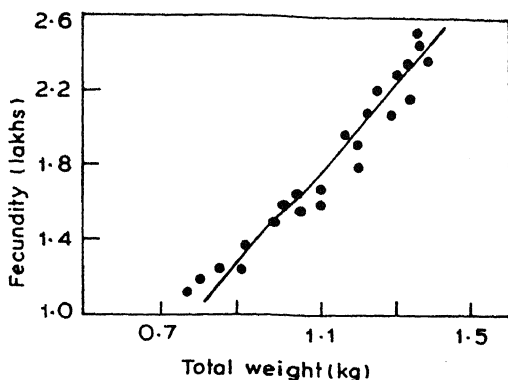
Figure 2. Relationship between fecundity and body weight in *H. molitrix*.
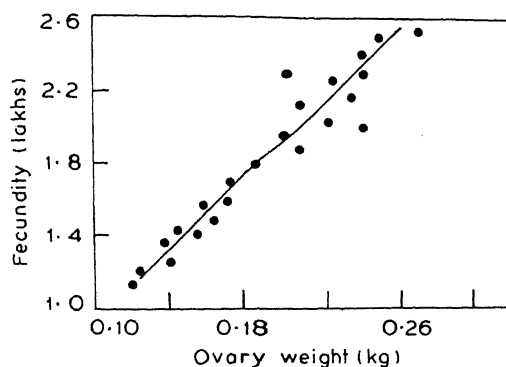
Figure 3. Relationship between fecundity and ovary weight in *H. molitrix*.

where FW is fish weight in kg. The analysis of variance proved the linearity of regression (observed $F = 15\cdot377$, significant at 1% level).

3.3 Fecundity and ovary weight

The relationship is expressed in figure 3. Egg production ranged from 1·14 lakh in an ovary of 120 g to 2·55 lakh in an ovary of 270 g. The fecundity-ovary weight relationship can be expressed as:

$$F = 0\cdot19352 + 8\cdot3839 \, OW \qquad (r = 0\cdot679453).$$

The analysis of variance proved the linearity of regression (observed $F = 8\cdot3$, significant at 5% level).

4. Discussion

Fish selected for the fecundity studies were more than two years old as the yearlings were removed from the dragnet. It has been observed that even the yearlings were

quite mature and ready for hypophysation. The original stock of silver carp fingerlings brought from Japan in 1959 started maturing when they were only 20 months old and attained full maturity by the time they were two years old. During 1962, when silver carp were 3 years old, they were induced to breed in ponds for the first time in India through hypophysation. The progeny so obtained became mature in ponds when they were only 11 months old (Alikunhi 1965).

The fecundity of fish has been studied by Clark (1934), Khan (1945), Alikunhi (1956), Varghese (1973), Joshi and Khanna (1980), Singh *et al* (1982) and Dobriyal and Singh (1987). In *H. molitrix*, the fecundity ranged from 1·14 lakh in a fish measuring 436 mm in total length, 760 g body weight and 120 g ovary weight to 2·55 lakh in a fish measuring 495 mm in total length, 1·350 kg body weight and 270 g ovary weight. The relative fecundity (fecundity/kg body weight) calculated for *H. molitrix* ranged from 1·48 to 1·78 lakh. Alikunhi *et al* (1963) reported the fecundity of silver carp, weighing 3·18–8·51 kg, as 1·45–20·44 lakh. The number of eggs per gram body weight were 171 and per gram ovary weight 292. Wu and Chung (1964) stated that the fecundity of pond reared breeders of Chinese carp in Kwangtung province, China, is high (1 lakh eggs/kg body weight). However, Kuronuma (1968) stated that silver carp from Tone river, Japan, treated with pituitary injections ranged from 9·5–11 kg in weight and fecundity from 10·98–13·92 lakh.

In *H. molitrix*, the fecundity increases with an increase in all the body parameters. A straight line relationship has been observed between the fecundity and fish length, fish and ovary weight. Clark (1934) suggested that the fecundity of a fish increased in proportion to square of its length. However, Simpson (1951) concluded that the fecundity of Plaice was related to cube of its length. A straight line relationship between the fish weight and fecundity has been reported by several workers (Bagenal 1957; Sarojini 1957; Varghese 1973). However, a curvilinear relationship has been reported by Varghese (1976) in *Coilia ramcarati*.

The study shows that the fecundity and body weight relationship was more closely related ($r = 0·8439$, $F = 15·377$ at 1% level of significance) than the fish length ($r = 0·704$, $F = 8·953$ at 5% level of significance) and ovary weight ($r = 0·6794$, $F = 8·3$ at 5% level of significance). The study will be much helpful in making the scientific arrangements for the successful hatching of the spawned eggs after hypophysation.

Acknowledgements

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daily. Dechlorinated tap-water was used as diluent. The average physico-chemical factors of water were pH 7·9; alkalinity 180 mgl^{-1}, total hardness 200 mgl^{-1}, electrical conductivity 1200 μmhos cm^{-1}, dissolved oxygen 6·3 mgl^{-1}, dissolved carbon dioxide 2·4 mgl^{-1} and temperature 28·5°C. Salts of metals dissolved in acidic water (HCl) were added to the ambient medium. Organisms were cultured in cement cisterns with the addition of cow-dung. *Daphnia lumholtzi* and *Cypris subglobosa* were collected through small plankton hand net and individuals were sorted out with a wide bore pipette. Tests were performed in 100 ml glass beakers with 10 organisms in each. Two replicates were run simultaneously with a control. Percentage mortality were transformed to LC$_{50}$ by the methods of Finney (1981) and relativity by Litchfield and Wilcoxon (1949).

3. Results

Tolerance levels in tables 1 and 2 show higher values in case of *Cypris* than in *Daphnia* indicating that the former is more resistant than the latter. The trend of toxicity in both the cases was Cd > Cu > Zn. The LC$_{50}$ of Zn for *D. lumholtzi* ranged from 0·44–10·74 mgl^{-1}, within the exposure durations spanning from 12–96 h. However, values were as high as 8·35–47·78 mgl^{-1} in case of *C. subglobosa*. Similar was the trend for Cu (0·009–0·083 and 0·277–13·58) and Cd (0·083–2·325 and 0·69–12·66 mgl^{-1}). The LC$_{16}$ − LC$_{84}$ is an approximation of rise in toxic levels between upper and lower asymptomate in toxicity curve on the concentration axis, above and below which sigmoid nature of curve gets disturbed. However, the range between this had regression equations as presented in tables 1 and 2 and the data were nonheterogenous. A few heterogenous data may be the result of eye traction errors in plotting straight line between concentration and probit mortality. Relativity was computed taking into consideration Cd as one unit in interaction with *Daphnia*. Relativity ranged from 1–46·53, highest being for *Daphnia* and Zn interaction. Toxicity patterns of various metals are also represented on concentration-time axes (figure 1).

4. Discussion

Cu is known to cause haemodilution, hyperglycemia, increase in haemopoiesis, decrease in plasma glutamic oxaloacetic transaminase activity, reduced antibody production leading to diseased fish followed by mortalities (Spear and Pierce 1979).

Tolerance of fresh water organisms may differ due to cationic actions on Ca^{+2} and Mg^{+2} levels. Suspended solids and colloids may form complexes with cupric ion and adsorption respectively thereby altering toxicities (Spear and Pierce 1979). In aqueous media, Cu ions co-ordinate with water molecules and form aqua-copper ions (Cu, H$_2$O$_6$)^2, in the absence of other interfering agents. The aqua ions of Cu get into animal tissues and bio-accumulates. However, the whole body concentrates of animals of higher trophic levels appear to have less metals. This may be because of higher metabolic efficiencies of these organisms. However, dynamics of any metal in a food-web is very little known. The earlier studies show that dissolved Cu converts itself into non-dissolved form within 5 days. Some of it get affected by water hardness, body tissue fluids leading to change in cell permeability (Lloyd 1962; Hoar 1969). Thus increase in fluid hardness decreases toxicity.

The toxicity values of the present study were found very similar to those stated in

Table 1. Tolerance levels of *D. lumholtzi* at various exposures with regressions, heterogeneity and relativity.

Test chemical	Exposure duration (h)	Tolerance levels in mgl^{-1} (fiducial limits in parentheses)			Regression equation $Y = mx + c$	Hetero-geneity d.f. $x^2 =$	Relativity
		LC$_{16}$	LC$_{50}$	LC$_{84}$			
Cu	12	0.089 (0.048–0.099)	0.083 (0.078–0.088)	0.0996 (0.094–0.105)	$Y = 12.61x + 5.01$	1.08	—
	24	0.0502 (0.043–0.058)	0.0672 (0.062–0.0728)	0.09 (0.079–0.102)	$Y = 7.83x + 5.12$	0.78	—
	48	0.038 (0.038–0.042)	0.0546 (0.049–0.06)	0.07836 (0.07–0.87)	$Y = 6.33x + 5.11$	0.70	—
	96	0.0014 (0.0037–0.0074)	0.0094 (0.009–0.0178)	0.0623 (0.0261–0.150)	$Y = 1.21x + 5.37$	8.98*	1.0
Cd	12	0.9069 (0.362–1.379)	2.325 (1.625–3.327)	8.228 (3.80–18.81)	$Y = 1.87x + 4.9$	1.18	—
	24	0.7816 (0.60–1.01)	1.585 (1.31–1.91)	3.312 (2.43–4.25)	$Y = 3.24x + 4.93$	2.23	—
	48	0.3216 (0.25–0.42)	0.6186 (0.52–0.74)	1.19 (0.92–1.54)	$Y = 3.50x + 5.03$	9.04*	—
	96	0.034 (0.018–0.068)	0.083 (0.061–0.114)	0.206 (0.14–0.31)	$Y = 2.53x + 5.32$	3.22	8.83
Zn	12	5.776 (4.51–7.2)	10.74 (9.12–12.64)	19.95 (15.11–26.35)	$Y = 3.69x + 4.91$	2.38	—
	24	0.91 (0.23–3.67)	6.704 (2.96–15.16)	210.8 (14.0–3059.0)	$Y = 0.66x + 4.91$	—	—
	48	0.3134 (0.11–0.86)	2.29 (1.42–3.69)	16.62 (5.37–51.45)	$Y = 1.15x + 4.93$	4.51	—
	96	0.22 (0.13–0.37)	0.4375 (0.34–0.56)	0.87 (0.65–1.17)	$Y = 3.34x + 5.39$	0.30	46.53

*Data found to be heterogenous.

Effect of copper, cadmium and zinc on fish-food organisms, *Daphnia lumholtzi* and *Cypris subglobosa*†

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Abstract. The results of static and acute bioassay studies on *Daphnia lumholtzi* (Cladocera) and *Cypris subglobosa* (Ostracoda) to the varying dosages of Cu, Cd and Zn were presented. The 96 h, LC_{50} values for *Daphnia* were found to be 0·009, 0·083, 0·437 and for *Cypris* 0·25, 0·687, 8·3 mgl^{-1} for Cu, Cd and Zn respectively. This indicates that *Daphnia* is very sensitive while *Cypris* has a good amount of resistance. The rank order for toxicity of 3 metals is Cu > Cd > Zn. The study indicates that the waters getting polluted by heavy metals even at low concentration would remove the population of fish-food organisms without directly effecting fish fauna, thus making water body unfit for fishery use.

Keywords. Cu; Cd; Zn; bioassay; *Daphnia*; *Cypris*; $LC_{16-50-84}$.

1. Introduction

Metal concentrations have been on increase in rivers, lakes and ponds causing threats to aquatic environments. Though Cu and Zn are considered the essential trace elements of the human body, they become toxic at higher concentrations. Cd, however, is not known to be present in living organisms.

Copper sulphate a potent algicide is transiently toxic (Sawyer 1970) and gets transformed into organically bound undissolved sedimented Cu on lake bottom. This produces deoxygenation in epilimnion by decrease in algal photosynthesis and increase in oxygen demand due to decomposition of organisms killed by Cu (Spear and Pierce 1979). Cd is not known to have such effects but is said to be the most hazardous metallic pollutant causing impairments in liver, kidney and spleen function, spinal deformities, anemia and death in fishes (Holcombe *et al* 1976). Toxicity of heavy metals to zooplankton were studied by Biesinger and Christensen (1972) and Baudouin and Scoppa (1974). The importance of daphnids as experimental animals is reviewed by Anderson *et al* (1948) and Biesinger and Christensen (1972). The studies on Indian plankters are limited and hence an attempt has been made to study the effect of Cu, Cd and Zn on *Daphnia* and *Cypris*. Zooplankton are extremely sensitive to metals in comparison to fish. Zooplankton is the primary food source for several species of the fish and also forms a basic factor in aquatic food chain.

2. Materials and methods

Acute and static bioassays were followed (APHA 1980). Test solutions were renewed

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Table 2. Tolerance levels of C. subglobosa at different exposures with regressions, heterogeneity and relativity.

Test chemical	Exposure duration (h)	Tolerance levels in mgl⁻¹ (fiducial limits in parentheses)			Regression equation $Y = mx + c$	Heterogeneity d.f. $x^2 =$	Relativity
		LC_{16}	LC_{50}	LC_{84}			
Cu	12	13·581 (13·12–13·38)	13·581 (13·46–13·72)	13·93 (12·70–5·29)	$Y=91·24x+4·92$	*	—
	24	9·187 (6·698–12·24)	12·20 (10·68–13·95)	16·21 (11·49–2·69)	$Y=8·07x+4·9$	0·006	—
	48	0·886 (0·42–1·83)	5·363 (3·52–8·18)	32·4 (9·99–15·2)	$Y=1·27x+4·63$	8·47	—
	96	0·0925 (0·0004–0·16)	0·2773 (0·21–0·35)	0·9356 (0·529–1·65)	$Y=1·89x+5·12$	0·65	1·0
Cd	12	4·62 (1·76–12·21)	12·66 (10·04–15·96)	34·67 (20·74–58·01)	$Y=2·27x+4·89$	0·10	—
	24	2·68 (1·76–4·06)	6·981 (3·48–13·98)	18·19 (12·68–26·08)	$Y=2·39x+5·12$	1·32	—
	48	2·32 (2·10–2·56)	3·02 (2·76–3·31)	4·49 (4·11–4·99)	$Y=6·93x+5·33$	*	—
	96	0·3646 (0·25–0·52)	0·6874 (0·55–0·92)	1·296 (0·95–1·77)	$Y=3·61x+5·55$	−5·08	2·48
Zn	12	40·59 (35·31–46·66)	47·78 (43·95–51·95)	56·26 (53·15–59·55)	$Y=14·02x+5·93$	18·10	—
	24	39·77 (34·68–45·6)	50·62 (46·71–54·86)	64·4 (56·80–73·05)	$Y=9·50x+4·98$	0·56	—
	48	21·36 (16·86–27·06)	34·99 (30·76–39·77)	57·27 (21·33–153·7)	$Y=4·64x+4·98$	8·16	—
	96	3·312 (2·09–5·05)	8·352 (6·5–10·71)	21·06 (14·46–30·70)	$Y=2·48x+5·21$	2·07	30·12

*Data found to be heterogenous.
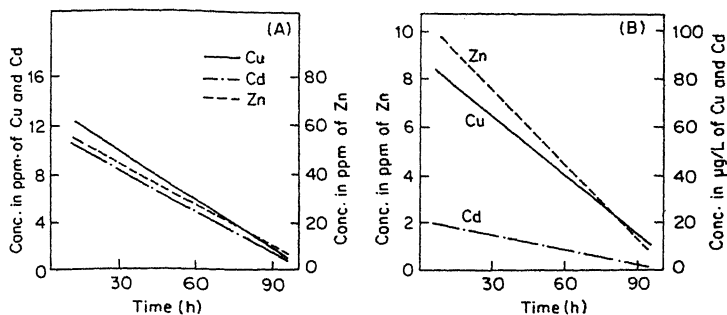
Figure 1. Toxicity relation of various metals to (A) *C. subglobosa* and (B) *D. lumholtzi* at LC_{50} levels.

the earlier works of Biesinger and Christensen (1972) and Winner *et al* (1977). Biesinger and Christensen (1972) studied 21 metals and concluded that Cu and Cd were the most toxic and Zn, median toxic to *Daphnia*. Effects of change in hardness and alkalinity seem nullified by effects of increased temperature (20–30°C).

Exposure of *Daphnia* to metallic toxicants reduces its own and progenial longevity significantly. It has also been observed that preadult invertebrates are generally less tolerant than adult (Spear and Pierce 1979). Stages, especially of ecdysis and pupa formation have been found more sensitive. Metals also promote ecdysis. These aberrations are related to enzyme inhibition by metals. Metals are also known to effect nervous system causing erratic swimming and loss of equilibrium in organisms residing in or exposed to polluted waters also observed in this study.

The present results have significance in manipulating the doses for algal eradication, detection of the pollution levels and hazard assessment in sub-temperate conditions of south-Rajasthan. Furthermore, results are significant in respect of the presence of zinc excavation and refining unit (Hindustan Zinc Ltd., Udaipur), nearby to the experimental site (10 km). This unit was established about a decade and a half ago and has by now almost destroyed the wild fauna and flora around its location and seriously threatened the nearby aquatic fauna by its fumes and acidic metal effluents. Recently, the company has undertaken antipollution measures like re-extraction of Zn from the wastes and liming the acidic effluents. However, the harm already done to the natural surroundings cannot be reversed.

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Glycosidases in toad (*Bufo melanostictus*) sperm and their role during fertilization

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Abstract. Sperms were isolated from *Bufo melanostictus* testes, by a simple technique of differential centrifugation. β-Galactosidase, N-acetyl β-D-glucosaminidase, α-L-fucosidase, neuraminidase and acid phosphatase activities were detected in the sperms. All the sugar components of the jelly glycoprotein were released·when toad jelly glycoprotein was treated with sperm suspension. These facts suggest that the sperm glycosidases are involved in the penetration of jelly by the sperm at the time of fertilization.

Keywords. *Bufo melanostictus*; glycosidase; sperm; fertilization.

1. Introduction

The eggs of most animals are surrounded by one or more coats or integuments through which a sperm must pass through to fertilize the egg (Rugh 1961; Monroy 1965; Metz 1967; Austin 1968; Piko 1969). They include the vitelline coat and jelly coats in many invertebrates (Wasserman *et al* 1985) and vertebrates (Wolf *et al* 1976) and zona pellucida in mammals (Srivatsava *et al* 1974; Urch *et al* 1985). These coats are composed mostly of glycoproteins with different ultra structures and molecular compositions (Katagiri 1987). The functions of these integuments are multiple such as participation in the process of sperm egg fusion, having specific binding sites for sperm or inducing acrosome reaction and also as a possible block of polyspermy (Gwatkin 1977; Glabe and Vacquier 1978; Suzuki *et al* 1981; Wasserman *et al* 1985).

The egg of an amphibian toad (*Bufo melanostictus*) is surrounded by a vitelline envelop and later on by the jelly which is produced in the oviduct when the egg passes through it. Jelly is an o-glycosidic type of glycoprotein containing N-acetyl glucosamine, N-acetyl galactosamine, fucose, galactose and N-acetyl neuraminic acid as its carbohydrate constituents. A partial structure for the carbohydrate moiety of the jelly has been proposed (Reddy and Seshadri 1978; Seshadri and Reddy 1980). These jelly envelops secreted by the oviduct are found to be indispensible for sperm penetration into eggs, as the removal of jelly layers resulted in the reduced fertilization efficiency (Reddy and Seshadri 1978). However, the exact mechanism by which the jelly coats favour fertilization is not clearly understood (Katagiri 1987).

Unlike most urodels (Katagiri 1987), fertilization in anuran amphibians is external. Fertilizing spermatozoa must pass through several layers of jelly envelops and the vitelline coat before fusing with the egg plasma membrane, a process known as acrosome reaction. Reports are available about the mechanism of passage of sperm

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through the vitelline envelop which reveal the involvement of acrosomal proteases
the lysis of vitelline coat. In amphibians, no reports are available about t
mechanism of cleavage of jelly layers. However, the possibility of the sperm conta
ing lytic activities other than proteases in order to accomplish fertilization has be
indicated (Katagiri 1987).

Shylaja and Seshadri (1985) have reported the presence of glycosidases such as
galactosidase, α-L-fucosidase, N-acetyl β-D-hexosaminidase, neuraminidase and a
proteases in toad (*B. melanostictus*) testes. These enzyme activities were found to v.
with respect to spermatozoa during breeding and non-breeding seasons. The pres
investigation was carried out with a view to find out whether the glycosidase acti
ties are present in the fertilizing sperms of the toad (*B. melanostictus*) and if pres
does this sperm uses these enzymes to cleave the egg coat jelly during its pass:
through it, which is an essential event for the sperm to reach the egg surface.

In this study, we report the isolation of sperms from toad (*B. melanostictus*) tes
the presence of glycosidases in sperms and their involvement in the degradation
the jelly.

2. Materials and methods

2.1 *Chemicals*

Para-nitrophenyl derivatives of β-D-galactopyranoside, α-L-fucopyranoside,
acetyl-β-D-glucosaminide and N-acetyl-β-D-galactosaminide, Fetuin, D-galacto
L-fucose and para-nitrophenyl phosphate were purchased from Sigma Chemi
Company, St. Louis, Missouri, USA. Dialysis tube (Cat. No. 3787H47) was purch
ed from Arthur H Thomas Company, Philadelphia, USA. Other chemicals used w
of analytical grade. All the solvents were distilled before use.

Locally available mature male toads (*B. melanostictus*) were collected dur
breeding season from their natural habitat. Testes were collected from the toads a
sacrificing the anaesthetised animals.

2.2 *Isolation of sperm*

About 20 g of freshly collected toad (*B. melanostictus*) testes were taken in 5 m
0·25 M sucrose solution or DeBoer's solution consisting of 110 mM Na
1·3 mM KCl and 1·3 mM CaCl$_2$ and finely chopped with razor blade to release
sperms. The material was then centrifuged at 80 g at 4°C for 10 min using 5C
Sorvall centrifuge. The debris devoid of sperms, as determined by the microsco
observation was discarded, and the supernatant was again centrifuged at 500 g
15 min at 4°C. The sperms sedimented as a pellet were washed several times v
0·25 M sucrose and later on with ice cold DeBoer's solution until the washings
free from protein and showed no enzyme activity and referred to as spe
suspension. This sperm suspension in DeBoer's solution was screened for vari
glycosidase activities.

To determine whether these enzymes are membrane bound or cytosolic,
washed sperms were suspended in DeBoer's solution and gently homogenized us
a potter Elvehjam blender using 30 strokes per min for 15 min. The homogenate v

centrifuged at 80,000 g for 30 min at 4°C. The supernatant was referred to as sperm extract. The sedimented fraction was washed thoroughly with ice cold saline and referred to as sperm membrane pellet. It was suspended in 2 ml of saline. Microscopic observation was carried out to make sure that the sperm extract is not contaminated with membrane fraction. In another experiment, the sperms suspended in DeBoer's solution were homogenized in the presence of 0·2% Triton-X-100 as above and centrifuged at 80,000 g for 30 min at 4°C. Both, supernatant and the sedimented sperm membrane pellets, were taken for enzyme assays.

The sperm extract and sperm membrane pellet were dialysed against water and the non diffusible portions were screened for various glycosidase activities.

2.3 Assay of glycosidases

β-D-Galactosidase, α-L-fucosidase and N-acetyl-β-D-hexosaminidase activities were assayed using corresponding para-nitrophenyl sugar derivatives as the substrate, according to the method of Alam and Balasubramanian (1978). Neuraminidase activity was assayed using toad jelly glycopeptide (prepared as described by Reddy and Seshadri 1978) or fetuin as the substrate and by estimating the released sialic acid by the method of Aminoff (1961). Acid phosphatase activity was assayed according to the method of Alvarez (1962), using para-nitrophenyl phosphate as the substrate. Protein was estimated by the method of Lowry *et al* (1951) using bovine serum albumin as standard.

2.4 Action of sperm glycosidases on toad oviduct jelly glycoprotein

In a dialysis tubing, 20 mg of toad jelly glycoprotein was treated with 2 mg protein of dialysed sperm extract and sperm membrane pellet separately. The reaction was carried out at 30°C by immersing the dialysis tubing in 30 ml of distilled water taken in a beaker. The dialysate obtained after 3 h incubation was evaporated to dryness under vacuum, dissolved in 0·1 ml of distilled water and analysed for the presence of various constituent sugars of jelly glycoprotein by paper chromatography as follows. The dialyzate sample (100 μl) was spotted on Whatman No. 1 paper and chromatographed using Butanol/pyridine/water (6:4:3 v/v) solvent system for 18 h, spots corresponding to different sugars were visualized on the paper using alkaline silver nitrate spraying reagent. Sialic acid was also visualized as described by Warren (1960). Jelly was also treated with sperm suspension under similar conditions.

2.5 Effect of sulphydryl group modifying reagents on sperm glycosidases

About 1 ml of sperm suspension (10^6 sperms/ml) in DeBoer's solution was treated with excess of 100 mM iodoacetate or N-ethyl maleimide at 37°C for 30 min according to the method of Colman and Chu (1970). The excess reagent was removed by dialysing the sperm suspension against water. The activity of glycosidases was determined using this sperm suspension, as described earlier.

3. Results

Microscopic observation of the washed sperm pellet revealed that it is not contaminated with other testicular cells (figure 1).

β-Galactosidase, α-L-fucosidase, N-acetyl-β-D-glucosaminidase, neuramini̇c̣
and acid phosphatase activities were detected in toad (*B. melanostictus*) sperms. '
above enzyme activities are found in the sperm extract as well as in the sp̣
membrane pellet but the specific activity of N-acetyl-β-D-glucosaminidase .
neuraminidase was higher in the sperm membrane pellet than in the sperm extṛ
whereas most of the acid phosphatase activity was found in the sperm pellet (tạ
1). However, when the sperm suspension was homogenized in the presence of dẹ
gents like Triton X-100, all the enzyme activities were found only in the sp̣
extract.
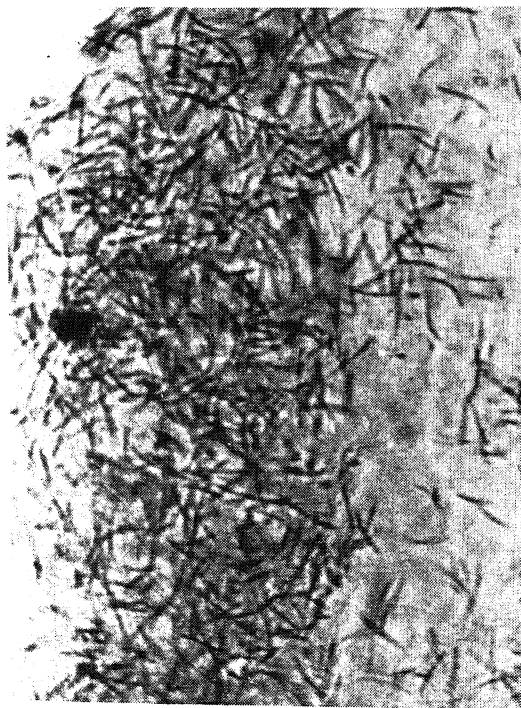
Figure 1. Isolated and washed sperms from toad (*B. melanostictus*) testes.

Table 1. Glycosidases in toad (*Bufo melanostictus*) sperm.

Source	Specific activity				
	N-acetyl-β-D glucos-aminidase[a]	β-Galacto-sidase[a]	α-L-Fuco-sidase[a]	Acid phos-phatase[a]	Neur-aminidase
Sperm extract	19·5 ± 1·8	13·1 ± 1·0	10·1 ± 1·5	2·0 ± 0·3	4·9 ± 0·8
Sperm membrane pellet	47·1 ± 2·0	5·8 ± 1·0	5·9 ± 1·0	70·7 ± 2·1	12·9 ± 1·3

The values represent the average of 4 separate experiments and expressed as mean ± SD.
[a]nanomol of para-nitrophenol released/mg protein/min.
[b]nanomol of sialic acid released/mg protein/min.

The sperm suspension, sperm membrane pellet and sperm extract hydrolysed the jelly glycoprotein. Silver nitrate positive spots corresponding to galactose, fucose, N-acetyl glucosamine and neuraminic acids were visualised on Whatman No. 1 paper (figure 2), indicating that the sperm glycosidases release the carbohydrate residues present in the jelly glycoprotein.

When the sperm suspension was treated with sulphydryl group modifying reagents, there was complete loss of all the glycosidase activities. This sperm suspension was unable to hydrolyse the jelly glycoprotein, as silver nitrate positive spots corresponding to constituent sugar residues of jelly glycoprotein were absent, after paper chromatography.

4. Discussion

Several methods are available in the literature about the isolation of sperms from mammals (Llanos *et al* 1982; Rahi *et al* 1983), however only little is known about
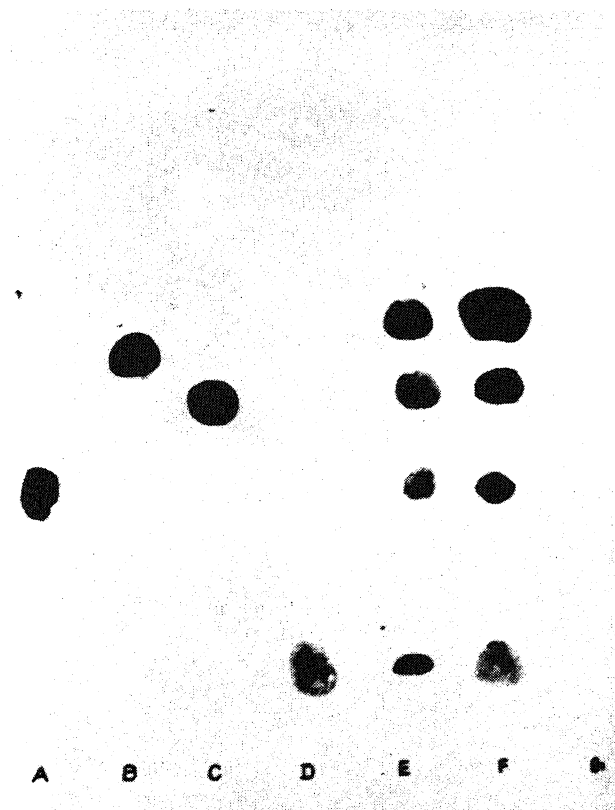
Figure 2. Effect of sperm glycosidases on toad egg coat jelly glycoprotein.
The isolated egg coat jelly was treated with toad sperm suspension and sperm membrane pellet separately and incubated in a dialysis tubing at 37°C for 3 h. The dialysate was collected after 3 h and evaporated to dryness in vacuo and analysed for jelly constituents by paper chromatography. A, Galactose; B, fucose; C, N-acetyl glucosamine; D, neuraminic acid; E, jelly treated with sperm membrane pellet; F, jelly treated with sperm suspension; G, control.

isolation of sperms from amphibians. In amphibians, the sperms are stored in testes which contain several specialized tissues and ejected only during spawning to accomplish fertilization. The fact that they have no specially differentiated organ like epididymis, as in higher organisms to store the spermatozoa; poses problems in isolation of sperms from amphibians. The present investigation describes a method for the isolation of sperms from toad (*B. melanostictus*) testes, adopting a simple technique of differential centrifugation which is very efficient and reproducible. Microscopic observation of the sperm suspension clearly showed that the method adopted for the isolation of sperms ensures that the sperm suspension is not contaminated with other cells present in the testes. This procedure can be adopted to isolate the sperms from the testes of other species of amphibians especially frogs.

There are many reports on the presence of glycosidases in mammalian sperm and their involvement in facilitating the fertilization (Allison and Hartree 1970; Srivatsava *et al* 1974), whereas no such reports are available in case of amphibians. However, there are evidences for acrosomal localization of proteases involved in the lysis of vitelline coat (Penn and Gredhill 1972; Elinson 1974; Iwao and Katagiri 1982), atleast in some species of amphibians. The involvement of certain lytic factors which are present in the sperm, for the degradation of jelly coat and the nature of these lytic factors are not clearly understood. The results of our experiments clearly demonstrate the presence of glycosidases such as β-galactosidase α-L-fucosidase, N-acetyl glucosaminidase and neuraminidase in toad sperms. This indicates that sperms contain a set of glycosidases required to breakdown the carbohydrate moieties of toad egg coat jelly glycoprotein.

When the toad egg coat jelly glycoprotein was treated with the toad sperm suspension galactose, fucose, N-acetyl glucosamine and sialic acid were released from the jelly glycoprotein indicating that the sperm glycosidases can use jelly glycoprotein as substrate. The sequential action of these sperm exo-glycosidases results in the dissolution of the jelly layer. The glycosidase activities were found both in sperm extract and sperm membrane pellet though their specific activities varied (table 1). However, when the sperm suspension was homogenized in the presence of detergents like Triton X 100, all the enzyme activities were found only in sperm extract which clearly indicates that these enzymes are bound to the sperm membrane. This fact indicates that the fertilizing sperm uses these jelly digesting enzymes during their passage through the jelly envelop. The presence of acid phosphatase activity in mammalian sperm is well known, though its role in fertilization is yet to be understood. In toad sperm also acid phosphatase activity was detected and from the present knowledge, its importance in facilitating the fertilization is not clear.

Treatment of the sperm suspension with the sulphydryl group modifying reagents like iodoacetate and N-ethyl maleimide resulted in the complete loss of glycosidases activity. This sperm suspension could not degrade jelly glycoprotein establishing that the jelly could be degraded only by these glycosidases during the passage of sperm through the jelly.

Thus, the present investigation clearly demonstrates that the sperm glycosidases are responsible for cleavage of the carbohydrate portion of jelly during its passage through the jelly in order to meet the egg.

Acknowledgements

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Steroidogenic cells in the testis of larva, pupa and adult eri silkworm, *Philosamia ricini*—A histochemical study

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Abstract. The testis formed of 4 follicles develops as a definitive organ on the first day of IV instar. The localization of Δ^5-3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase and 11β-hydroxysteroid dehydrogenase, was limited to peritoneal sheath and epithelial layer of the testis of IV and V instar larva. From 3rd day of pupation upto the 8th day of pupation the enzyme activity extended to the cysts containing spermatogonical cells, primary and secondary spermatocytes and subsequently to the spermatids and spermatozoan bundles from 9th day of pupation and continued thereafter in the adults. Glucose-6-phosphate dehydrogenase activity was observed in all the testicular components during larval, pupal and adult stages. These results indicate that the developing and adult testis of eri silkworm, *Philosamia ricini* has the ability to metabolize hydroxysteroids.

Keywords. Eri silkworm; testis; spermatogenesis; steroidogenic cells.

1. Introduction

In recent years vertebrate type of hormonal steroids are shown to be present in arthropods, molluscs and echinoderms (Lehoux and Sandor 1970; Thompson *et al* 1973; Sandor *et al* 1975). In the last few years evidence has been accumulated to show that the gonads of insects are capable of metabolizing enzyme systems (Lehoux *et al* 1968; Lehoux and Sandor 1969; Dube and Lemonde 1970). *In vitro* conversion of Δ^5-3β-hydroxysteroids by the testis of cockroaches, *Gramphadorhina portentosa*, *Byrostria fumigata* and the cricket, *Gryllus assimilis* (Lehoux and Sandor 1970) has indicated, albeit indirect, the existence of Δ^5-3β-hydroxysteroid dehydrogenase (HSDH) activity and in fact it has been histochemically demonstrated in the testis of these insects (Lehoux and Sandor 1970) and in the testis of two hemipterans, *Graphosoma italicum* and *Eurydema ventralis* (Trandaburu and Tasca 1976). To the best of our knowledge there is no report on the histochemical demonstration of 17β-HSDH and 11β-HSDH in the testis of insects.

In the present investigation an attempt has been made to present histochemical evidence for steroid metabolizing potential of the testis of eri silkworm, *Philosamia ricini* during IV and V instar larval period, pupal period and in the adult before and after mating by demonstrating the presence of Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity. In addition, the histochemical localization of glucose-6-phosphate dehydrogenase (G-6-PDH) in the testis of this silkworm, is also carried out.

2. Materials and methods

The larva, pupa and adult *P. ricini* reared in the laboratory were used in the present investigation. For histochemical study the animals from respective stages of larva,

pupa and adult were decapitated, the testes were removed and immediately frozen over dry ice vapour at $-50°C$ and sectioned in a cryostat at $-20°C$. For the histochemical demonstration of Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity the frozen sections were incubated in the media containing different substrates (table 1) prepared according to the procedure described by Baillie *et al* (1966) and Saidapur and Nadkarni (1972). For the localization of G-6-PDH activity, the frozen sections were incubated for 15 min at 37°C in the medium prepared according to the methods described by Bara (1965a, b). After incubation the sections were washed in distilled water, fixed in 10% neutral formalin for 30 min and mounted in glycerol jelly. Parallel sections incubated in the media lacking the substrates served as controls. A few sections incubated in the medium containing dehydroepiandrosterone (DHA) co-enzyme, nitro blue tetrazolium (NBT) and cyanoketone/isoxozol, as specific control of Δ^5-3β-HSDH activity.

3. Results and discussion

The results on the histochemical reaction for the HSDH, indicated by the deposition of blue diformazan granules in the cytoplasm of different cells types of the testis of larva, pupa and adult *P. ricini* are summerised in table 1.

A weak Δ^5-3β-HSDH activity was observed only in the cells of the peritoneal sheath and epithelial layer of the testis (figure 1) of IV and V instar larva of *P. ricini*. Of the two substrates used, pregnenolone was somewhat better utilized than DHA.

On the first day of pupation the spermatogoneal cells showed activity in traces, the primary and secondary spermatocytes did not show any activity (figure 2). From third day of pupation and onwards upto 12th day of pupation the cells of peritoneal sheath and epithelial layer showed moderate activity with DHA as the substrate and on 15th day of pupation the activity was intense with both the substrates (figure 3). The spermatogoneal cells showed a weak activity from 3rd day pupation to the remaining period of the pupal life. The primary and secondary spermatocytes, spermatids and spermatozoan bundles at the respective developmental stages of pupa showed a gradual increase in the intensity activity from traces to moderate (table 1).

A fairly intense Δ^5-3β-HSDH activity was observed in the cells of the peritoneal sheath and epithelial layer, spermatogonial cells, primary and secondary spermato-cytes, spermatids and spermatozoan bundles of the testis of adult *P. ricini* before mating (figure 4).

The distribution of 17β-HSDH activity with testosterone and 17β-estradiol in the cells of the peritoneal sheath and epithelial layer of the testis of IV and V instar larvae was similar to that of Δ^5-3β-HSDH activity in the corresponding instars.

There was a gradual increase of 17β-HSDH activity from moderate to intense in the cells of peritoneal sheath, epithelial layer and spermatogoneal cells from 3rd day of pupation upto 9th day of pupation. On 12th and 15th days of pupation the acti-vity was reduced to moderate with both the substrates.

On the 3rd day of pupation, the 17β-HSDH activity was in traces in primary and secondary spermatocytes and there was no activity in the spermatids. On 6th day of pupation the activity was moderate in primary and secondary spermatocytes and in spermatids. In the spermatozoan bundles the activity was in traces. On 9th, 12th and 15th day of pupation the activity was moderate in primary and secondary spermatocytes, spermatids and spermatozoan bundles (figure 5). There was relatively

Table 1. Activity of hydroxysteroid dehydrogenases in the testis of larva, pupa and adult *P. ricini*.

Developmental stages	Enzymes and the substrates[a]	Intensity of reaction[b]			
		Peritoneal sheath and epithelial cells	Spermatogonial cells	Primary and secondary spermatocytes	Spermatids and spermatozoan bundles
IV instar larva	Δ^5-3 β-HSDH				
1st and 3rd day	DHA	+ +	−	Not yet formed	Not yet formed
	Pregnenolone	+ + +	−	–do–	–do–
	17 β-HSDH				
	Testosterone	+ +	−	–do–	–do–
	17 β-estradiol	+ +			
	11 β-HSDH				
	11 β-hydroxyandro-stenedione	+ +	−	–do–	–do–
V instar larva					
1st day	Δ^5-3 β-HSDH				
	DHA	+ +	−	Only Primary	–do–
	Pregnenolone	+ + +	−	spermatocytes	–do–
	11 β-HSDH				
	11 β-hydroxyandro-stenedione	+ +	−	−	–do–
	17 β-HSDH				
	Testosterone	+ +	−	−	–do–
	17β-estradiol	+ +	−	−	–do–
3rd day	Δ^5-3 β-HSDH			Primary and	
	DHA	+ +	−	secondary	Not yet formed
				spermatocytes	
	Pregnenolone	+ + +	−	−	
	17 β-HSDH				
	Testosterone	+ +	−	−	–do–
	17β-estradiol	+ +	−	−	–do–
	11 β-HSDH				
	11 β-hydroxyandro-stenedione	+ +	−	−	–do–
Pupa					
1st day	Δ^5-3 β-HSDH				
	DHA	+ +	±	−	–do–
	Pregnenolone	+ + +	±	−	
	17 β-HSDH				
	Testosterone	+ +	±	−	–do–
	17β-estradiol	+ +	±	−	
	11 β-HSDH				
	11 β-hydroxyandro-stenedione	+ +	±	−	–do–
3rd day	Δ^5-3 β-HSDH				
	DHA	+ + +	+ +	±	–do–
	Pregnenolone	+ + + +	+ +	±	
	17 β-HSDH				
	Testosterone	+ + +	+ +	+	–do–
	17β-estradiol	+ + +	+ +	±	
	11 β-HSDH				
	11β-hydroxyandro-	+ + +	+ +	±	–do–

Table 1. (*Contd.*)

Developmental stages	Enzymes and the substrates[a]	Intensity of reaction[b]			
		Peritoneal sheath and epithelial cells	Spermatogonial cells	Primary and secondary spermatocytes	Spermatids and spermatozoan bundles
6th day	Δ^5-3β-HSDH				
	DHA	+ + +	+ +	+ +	+
	Pregnenolone	+ + + +	+ +	+ +	+
	17β-HSDH				
	Testosterone	+ + +	+ +	+ +	+ +
	17β-estradiol	+ + +	+ +	+ +	+
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ + +	+ +	+ +	+
9th day	Δ^5-3β-HSDH				
	DHA	+ + +	+ +	+ + +	+ + +
	Pregnenolone	+ + + +	+ +	+ + +	+ + +
	17β-HSDH				
	Testosterone	+ + +	+ + +	+ + +	+ + + +
	17β-estradiol	+ + +	+ + +	+ + +	+ + +
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ + +	+ + +	+ + +	+ + +
12th day and 15th day	Δ^5-3β-HSDH				
	DHA	+ + +	+ +	+ + +	+ + +
	Pregnenolone	+ + + +	+ +	+ + +	+ + +
	17β-HSDH				
	Testosterone	+ + +	.+ +	+ + +	+ + + +
	17β-estradiol	+ + +	+ +	+ + +	+ + +
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ + +	+ +	+ + +	+ + +
Adult *Before mating*	Δ^5-3β-HSDH				
	DHA	+ + +	+ + +	+ + +	+ + +
	Pregnenolone	+ + + +	+ + +	+ + +	+ + +
	17β-HSDH				
	Testosterone	+ + +	+ + +	+ + +	+ + + +
	17β-estradiol	+ + +	+ + +	+ + +	+ + +
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ + +	+ + +	+ + +	+ + +
After mating	Δ^5-3β-HSDH				
	DHA	+ +	+ +	+ +	+ + +
	Pregnenolone	+ + +	+ +	+ +	+ + +
	11β-HSDH				
	Testosterone	+ +	+ +	+ +	+ + +
	17β-estradiol	+ +	+ +	+ +	+ +
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ +	+ +	+ +	+ + +
	Control	−	−	−	−

[a]All the chemicals are of Sigma grade, obtained from Sigma Co., USA.
[b]Intensity of reaction is graded from minimum (+) to intense (+ + + +) activity, (−) denotes absence of reaction and (±) denotes trace activity.
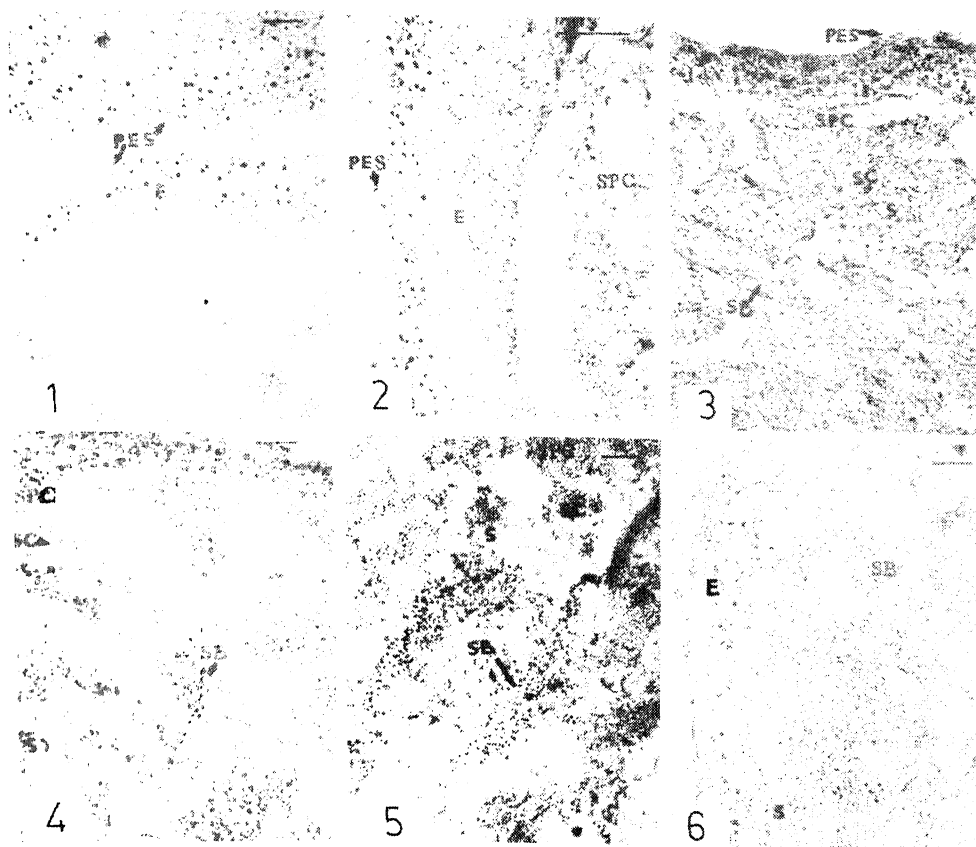
Figures 1–6. **1.** Δ^5-3β-HSDH activity (dark granules) in the cells of the peritoneal sheath (PES) and epithelial layer (E), in the fresh frozen section of the testis of IV instar larva of *P. ricini* with DHA as the substrate. **2.** Δ^5-3β-HSDH activity in the cells of the peritoneal sheath (PES), epithelial layer (E) and the spermatogoneal cells (SPC) in the fresh frozen section of the testis of one day old pupa of *P. ricini* with pregnenolone as substrate. **3.** Δ^5-3β-HSDH activity in the cells of the peritoneal sheath (PES), epithelial layer (E), spermatogoneal cells (SPC), spermatids (S) and spermatozoan bundle (SB), in fresh frozen section of the testis of 15 day old pupa of *P. ricini* with pregnenolone as substrate. **4.** Fairly intense Δ^5-3β-HSDH activity in the epithelial layer (E), spermatogonial cells (SPC), spermatids (S) and spermatozoan bundles (SB) in fresh frozen section of the testis of adult *P. ricini* before mating, with DHA as substrate. **5.** 17β-HSDH activity in the spermatids (S) and spermatozoan bundles (SB) in fresh frozen section of the testis of 12 day old pupa of *P. ricini* with 17β-estradiol as substrate. **6.** A weak 17β-HSDH activity in the epithelial layer (E), spermatids (S) and spermatozoan bundles (SB) in fresh frozen section of the testis of adult *P. ricini* after mating with testosterone as the substrate.

The scale in the figures indicate 40 μm.

more diformazan granules in the sperm bundles when testosterone was used as the substrate, indicating that the spermatozoan bundles seemed to have utilized testosterone better than 17β-estradiol.

In the testis of adult *P. ricini* the spermatozoan bundles utilized testosterone better than 17β-estradiol. The reaction in spermatogonial cells, primary and secondary spermatocytes, spermatids and spermatozoan bundle of adult after mating was reduced to moderate activity (figure 6).

The intensity and distribution of 11β-HSDH activity with 11β-hydroxyandrostenedione as the substrate, in the various components of the testis of IV and V instar larvae and on the day of investigation during the pupal period (1st, 3rd, 6th, 9th, 12th and 15th day) and in the adults, before and after mating in *P. ricini* (table 1) was similar to that of 17β-HSDH activity in the corresponding periods.

An intense G-6-PDH activity was observed in all the components of the testis in larva, pupa and adult of *P. ricini*. After mating a slight reduction in the enzyme activity was observed in all the testicular components except the cells of peritoneal sheath and epithelium of the testis.

The control sections incubated in the medium lacking either the substrate or the co-enzyme did not give any positive reaction but some times a very weak reaction was observed. The control sections treated with cyanoketone or isoxozol prior to incubation and incubated in normal medium containing DHA, did not give any histochemical reaction, thus indicating the specificity of Δ^5-3β-HSDH activity.

The steroid biosynthesis is not an exclusive prerogative of vertebrates, since steroid biosynthesis is wide spread throughout the animal kingdom (Barrington 1968). The histochemical demonstration of Δ^5-3β-HSDH activity is generally taken as an evidence, albeit indirect, that the particular tissue is capable of steroid metabolism or biosynthesis (Lehoux and Sandor 1970). *In vitro* and histochemical studies by Lehoux and Sandor (1970) have shown the occurrence of Δ^5-3β-HSDH in the testis of cockroaches, *G. portentosa*, *B. fumigata* and the cricket, *G. domesticus*. However, there is no detailed information as to which of the testicular components showed Δ^5-3β-HSDH activity. Recently Trandaburu and Tasca (1976) have histochemically demonstrated the presence of Δ^5-3β-HSDH activity in the spermatids and spermatozoan of two heteropteran species, *G. italicum* and *E. ventralis*. In the present investigation the cells of the peritoneal sheath and epithelium are the first testicular components to possess the Δ^5-3β-HSDH activity.

The occurrence of Δ^5-3β-HSDH in the spermatids and spermatozoan bundles in the testis of this insect is in conformity with that of *G. italicum* and *C. ventralis* (Trandaburu and Tasca 1976). The reduction of Δ^5-3β-HSDH activity in all the components of the adult after mating indicate the reduced metabolic rate of steroid hormones. In the present investigation occurrence of Δ^5-3β-HSDH in the testicular components during larval, pupal and adult stages, it may be suggested that the testis of developing and adult *P. ricini* possess the enzyme or enzymes necessary to convert exogenous pregnenolone to progesterone and DHA to androstenedione.

17β-HSDH catalyses the interconversion of androstenedione testosterone and 17β-estradiol-estrone in the gonads of vertebrates (Baillie *et al* 1966). 17β-HSDH enzyme system has been isolated from the various nonreproductive tissues and testis of *G. domesticus* (Lehoux and Sandor 1969) and the ovary and testis of *S. gregaria* (Dube and Lemonde 1970). In these studies the enzyme activity was shown to be highest in the male and female gonads on tissue weight basis. 17β-HSDH activity has also been histochemically demonstrated in the ovary of *Bombyx mori* and *Antheraea mylitta* (Hurkadli *et al* 1988).

In the present investigation a weak 17β-HSDH activity in the various components of the testis of IV and V instar larva of *P. ricini* indicates a weak metabolism of sex steroids. The activity of this enzyme was gradually extended to the spermatogonial cells, primary and secondary spermatocytes, spermatids and spermatozoan bundles of the testis during pupal period. At the same time even the intensity of reaction was also increased. These results indicate the increased quantity of 17β-HSDH activity

being present in these tissues during latter half of pupal period. This in turn may result in the increased rate of sex steroid metabolism. In the last few days of pupal period, the activity of this enzyme was maximum which may be correlated to the increased metabolism of testosterone and 17 β-estradiol by spermatozoans during that period. 17 β-HSDH activity found in the various components of the testis of adult after mating is reduced which may be due to cessation of spermatogenic activity of the testis. Our histochemical findings, that the testis of *P. ricini* contains 17 β-HSDH activity is in conformity with the biochemical investigations of other workers (Lehoux and Sandor 1969, 1970; Ohnishi 1985). The present results indicate that the testis of larva, pupa and adult *P. ricini* possesses 17 β-HSDH activity which can bring about the interconversions of testosterone-androstenediol and 17 β-estradiol-estrone. It may be suggested that the testis of *P. ricini* has the capacity to metabolize the sex steroids.

The occurrence of corticosterone and cortisol in the haemolymph of the cricket *G. domesticus* has been reported (Lehoux and Sandor 1970). The ovaries and pyloric caeca of the star fish, *A. rubens* are known to produce *11*-desoxycortosterone (Schoenmakers and Voogt 1980). 11 β-HSDH activity has been histochemically demonstrated in the ovaries of *B. mori* and *A. mylitta* (Hurkadli *et al* 1988). In the present investigation 11 β-HSDH activity has been demonstrated in the testis of larva, pupa and adult *P. ricini* and this indicates the ability of the testis to metabolize 11 β-hydroxysteroids.

G-6-PDH provides energy needed for hydroxylation during steroidogenesis or steroid metabolism in steroidogenic tissues of vertebrates (McKerns 1968). G-6-PDH activity indicates the presence of NADPH generating system in the testis of developing and adult *P. ricini*.

In conclusion the present investigation indicates that the testis of the larva, pupa and adult *P. ricini* has the necessary steroid converting enzymes that can convert the exogenous hydroxysteroids to ketosteroids.

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Effect of leaf ration on dietary water budget of the larvae of silkworm *Bombyx mori* and eri silkworm *Philosamia ricini*

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Abstract. Dietary water intake in penultimate two instars of silkworm *Bombyx mori* and eri silkworm *Philosamia ricini* fed *ad libitum* on mulberry leaf *Morus alba* and castor leaf *Ricinus communis* respectively at $26 \pm 2°C$ is reported. The lower ration level results in accumulation of water content in the body of insects. The water retention efficiency (per cent of absorbed water retained in the body) increases with the decreasing ration level. The larva retains higher amount of water in the body by increasing water retention efficiency from 22% at 100% ration to 61% at 25% ration. This is an important adaptive mechanism exhibited by larvae. The water utilization budgets are discussed with the budgets available for other insects.

Keywords. Dietary water; water retention efficiency; *Bombyx mori*; *Philosamia ricini*.

1. Introduction

The importance of water in relation to food intake and utilization has been stressed by many authors (Michael *et al* 1971; Reese and Beck 1978; Scriber and Slansky 1981; Delvi 1983; Reddy 1981). Water is a very important component of insect diet (Ross 1956) particularly in the leaf feeders like the *Bombyx mori* and *Philosamia ricini*, which meet their water requirement from their food (Delvi and Naik 1984).

There are few reports concerning the dietary water balance and the effects of environmental factors like temperature, leaf ration (Pandian *et al* 1978; Delvi 1983) and insecticides (Naik and Delvi 1984). The water gain and loss during ingestion and digestion of food and mechanisms regulating them have not been studied adequately in economically important insects of *B. mori* and *P. ricini*. This communication reports on the effect of ration levels on water budget of penultimate two instars of *B. mori* and *P. ricini*.

2. Material and methods

Egg layings of *B. mori* (pure Mysore race) were obtained from the grainage of the Government of Karnataka (Magadi area) and eggs of *P. ricini* were obtained from University of Agricultural Sciences, GKVK Campus, Bangalore and maintained in the laboratory. Freshly hatched larvae were removed to separate terraria (plastic trays size $36 \times 26 \times 4$ cm). The larvae were maintained in the laboratory fed on leaves of *Morus alba* and *Ricinus communis* respectively at room temperature and humidity which averaged $26 \pm 2°C$; $80 \pm 10\%$ RH. Lepidopteran larvae consumed more than 97–98% of the total food intake during the final two instars (Waldbauer 1968). Hence, the effect of ration level on water consumption and utilization was estimated in the penultimate two instars in *B. mori* and *P. ricini*. The test individuals were

reared in 3 groups (each consisting of 50 larvae) and experiments were triplicated in each group. The larvae were fed on fresh leaves of *M. alba* and *R. communis* at 100, 75, 50 and 25% ration levels. The ration levels were fixed considering the food consumption values of *B. mori* and *P. ricini* fed *ad libitum* separately (see also Naik 1986).

Fresh leaves of *M. alba* and *R. communis* were cut into two halves, one half was weighed and offered to larvae and the other was used as control to find the initial water content (Delvi and Pandian 1972). Larvae began to feed as soon as the leaf was offered. Since they fed throughout the day, the fresh leaves were offered 4 times a day to minimise the amount of water evaporated from the leaves. Amount of daily food intake was calculated following the standard gravimetric method described by Waldbauer (1968), Delvi (1983), Naik and Delvi (1984) and Delvi and Naik (1984).

Some insects are known to obtain atmospheric water through the integument (Bodine 1921; Lugwig 1937; Beament 1964) and in few cases via cloacal ends (Beament 1961) or via the spiracles (Buxten 1932). *B. mori* and *P. ricini* should have exchanged a considerable amount of water with ambient air; however, this was not estimated (see also Uvarov 1966; Delvi 1983). The absorption of atmospheric water and transpiration of body water through integument have been dealt in detail by previous workers (Wigglesworth 1957; Edney 1957, 1967; Ebeling 1964; Cloudsley-Thompson 1962; Beament 1964). Perhaps *B. mori* and *P. ricini* meets almost complete required water from the ingested leaf of *M. alba* and *R. communis* (see also Pandian *et al* 1978; Delvi 1983).

3. Results

The initial water content of the larvae chosen for the experiments changed little and averaged $77.5 \pm 0.2\%$ in *B. mori* and $82 \pm 1.2\%$ in *P. ricini* immediately after the completion of third instar (table 1). At pupation water content of the terminal *B. mori* larvae decreased to 67% during 100 and 75% feeding. However, the decreased ration level resulted in increased water content of 86·5 at 50% ration level and 90% at 25% ration level in *B. mori*. Such differences in the final water content of the larvae are not found in *P. ricini*, where the water content change little with different ration levels and averaged about 78%. Perhaps less percentage of water found in the fifth instar larvae of *B. mori* during 100 and 75% ration levels may be due to the fact that

Table 1. Effect of ration feeding on the water content of the larvae and excreta in *B. mori* and *P. ricini*.

Ration (% of ad libitum =100%)	B. mori					P. ricini				
	Water content of the larvae			Water content of the excreta		Water content of the larvae			Water content of the excreta	
	IV Instar	V Instar	Final day	IV Instar	V Instar	IV Instar	V Instar	Final day	IV Instar	V Instar
100	74·4	84·34	66·8	34·42	34·42	81·5	79·9	78·5	22·9	37·3
75	77·5	82·54	67·0	34·42	34·42	82·8	81·3	77·4	16·9	28·9
50	77·6	87·8	86·5	19·2	39·4	82·3	78·6	77·8	14·7	25·6
25	77·5	88·9	89·9	16·7	38·6	81·3	79·9	78·2	19·3	31·9

excess amount of water was lost through the faeces during the fifth instar period.

The water content of freshly defaecated pellets in *B. mori* reduced initially in the fourth instar larvae depending on the ration level; the 100 and 75% feeding exhibited no significant difference in the water content of excreta and averaged 34·4%. A change in the ration level to 50% reduced the water content of excreta to 19·2% and further to 16·7% at 25% ration level. However, during fifth instar, the water content of the faeces range from 34·4–39·4% in all the groups of *B. mori*. In *P. ricini* a similar trend of water content of the excreta is found.

With the decreasing ration level, the amount of water retained in the body by the test individuals decreased. The larvae of *B. mori* consumed 5603 mg of water at 100% ration level; of this 85·4% was absorbed and 14·6% lost with faeces. A part of the absorbed water is retained in the body which amount to 1012 mg which is about 21·8% of the absorbed water. This water retention efficiency (per cent of absorbed water retained in the body) increases with the decreasing ration level. The efficiency was 27·6% with 75% ration, 44% with 50% ration and 61% with 25% ration (table 2). The decreasing ration level reduces the amount of water available to each insect. The water input is uniformly absorbed with the maximum efficiency of about 85% at all the tested ration levels. However, *B. mori* larva retained higher amount of water in the body by increasing water retention efficiency from about 22% at 100% ration to 61% at 25% ration. This is an important adaptive potential exhibited by the larvae. Similarly *P. ricini* larvae also increased the water retention efficiency with the decreasing ration levels. *P. ricini* absorbs the water with an efficiency ranging from

Table 2. Effect of ration levels on water utilization in final two instars of *B. mori* at 26 ± 2°C; 80 ± 10% fed *ad libitum* mulberry leaf *M. alba*.

Parameters	Ration			
	100%	75%	50%	25%
Larval period (day)	16·00	19·00	18·00	19·00
Dietary water intake (mg/insect)	5602·75	4453·60	3559·81	2133·10
Water loss through faeces (mg/insect)	971·56	879·10	645·68	377·36
Water absorbed (mg/insect)	4631·14	3574·50	2914·13	1755·74
Water retained in the body (mg/insect)	1011·80	985·42	1281·90	1070·70
Water loss through transpiration (mg/insect)	3619·34	2589·08	1632·22	685·04
Water intake rate (mg/mg/day)	0·607362	0·399158	0·348643	0·221054
Water loss rate through faeces (mg/mg/day)	0·081589	0·065795	0·036926	0·025188
Water absorption rate (mg/mg/day)	0·525973	0·258472	0·311718	0·195867
Water transpiration rate (mg/mg/day)	0·410061	0·243669	0·196385	0·0489711
Water absorption efficiency (%)	85·36	82·90	87·28	87·05
Water retention efficiency (%)	21·80	27·60	44·00	61·00

89–94% at different ration levels. The water retention efficiency increases from 32·5% at 100% ration level to 44·5% at 25% ration level (table 3).

Though the food offered was regulated at different ration levels after knowing the amount of food consumed fed *ad libitum*, the water intake was found to increase from 4–13% in *B. mori* and from 4–20% in *P. ricini* at different ration levels. This may be due to the increase in larval period at the decreased ration levels (tables 2 and 3). The water intake rate fluctuates widely in larvae receiving different rations (tables 2 and 3). The rate is higher during the fourth instar than the fifth at all the ration levels. Individuals of *B. mori* offered 100% ration display a higher water intake rate of 0·607362 mg/mg live weight of insect/day. Although the total water intake rate of individuals with 100 and 75% rations is higher than those fed with 50 and 25% rations, the latter fed actively while the former fed slowly at different intervals.

Both *B. mori* and *P. ricini* exhibited decreased rate of water loss through faeces with the increasing ration levels. The transpiration rate decreases steadily with decrease in the ration level. However the efficiency of water absorption changed little in both the test insects and averaged 85·6% in *B. mori* and 91·6% in *P. ricini*.

4. Discussion

Ingestion of water via food and subsequent absorption of water decreases with the decrease in ration levels in both *B. mori* and *P. ricini*. Dietary water intake or water absorbed steadily decreased from a maximum 5602·7 or 4631·1 mg at 100% ration to

Table 3. Effect of ration levels on water utilization in the final two instars of *P. ricini* at $26 \pm 2°C$; $80 \pm 10\%$ fed *ad libitum R. communicus*.

Parameters	Ration			
	100%	75%	50%	25%
Larval period (day)	7·00	9·00	9·50	11·00
Dietary water intake (mg/insect)	13181·30	10537·35	9230·81	5761·95
Water loss through faeces (mg/insect)	1979·58	1130·194	732·98	650·779
Water absorbed (mg/insect)	11200·98	9407·17	8497·92	5111·18
Water retained in the body (mg/insect)	3649·01	3274·83	2694·32	2273·26
Water loss through transpiration (mg/insect)	7551·06	6132·35	5803·54	2837·92
Water intake rate (mg/mg/day)	0·898819	0·74023	0·785319	0·516784
Water loss rate through faeces (mg/mg/day)	0·073486	0·052142	0·044872	0·0412611
Water absorption rate (mg/mg/day)	0·825318	0·68809	0·73786	0·4778403
Water transpiration rate (mg/mg/day)	0·524684	0·424189	0·502289	0·293431
Water absorption efficiency (%)	89·23	92·21	93·95	92·07
Water retention efficiency (%)	32·58	34·81	31·71	44·48

2133·1 or 1755·7 mg at 25% ration in *B. mori*. In *P. ricini* the decrease in the water intake or absorption ranges from 13181·3 or 11200·98 mg at 100% ration to 5761·9 or 5111·2 mg at 25% ration. The reduced ration levels (75, 50 and 25%) resulted in reduction in intake and utilization of water in both the test insects. The qualitative and quantitative importance of water with negative effects has been observed by several workers (Delvi and Naik 1984; Naik and Delvi 1984; Radhakrishna and Delvi 1987). A survey of relevant literature reveals the paucity of information regarding the effects of ration levels on water utilization. Though there are several papers on effects of other environmental factors like temperature (Delvi 1983; Pandian *et al* 1978), insecticides (Delvi and Naik 1984; Naik and Delvi 1984), the only paper that is available on the effect of leaf ration on the water budget is that of Pandian *et al* (1978). Delvi (1983) and Pandian *et al* (1978) have given valuable data on the water utilization of a lepidopteran insect *Danaus chrysippus*. In *D. chrysippus* with increasing ration, there was considerable increase in the dietary water intake, absorption and retention (Pandian *et al* 1978). In *B. mori* and in *P. ricini* the increasing ration level resulted in increased dietary water intake and utilization; the larvae fed 100 and 75% ration ingested and absorbed more water than the group receiving 50 and 25% ration. It has been demonstrated that *D. chrysippus* economises the water loss through reduced transpiration at lower rationing (Pandian *et al* 1978). It can be seen from tables 2 and 3 that ration levels had no influence on water absorption efficiency in both *B. mori* and *P. ricini* which retained around 85–90%. However, the water retention efficiency increases with the decreasing ration level; from 18% at 100% ration to 50% with 25% ration in *B. mori* and from 32·5% at 100% ration to 44·5% at 25% ration in *P. ricini*. This observation suggests that the lepidopteran larvae manage to get more water either by increasing the efficiency of water absorption (Pandian *et al* 1978; Delvi 1983) or by increasing the water retention efficiency (present work) during any unfavourable conditions. The conclusion is significant in the light of understanding the adaptive potential of the terrestrial insects.

Acknowledgements

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Effect of triol and makisterone A on the haemocytes of *Hieroglyphus nigrorepletus* Bolivar (Orthoptera: Acrididae)

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Abstract. Fifth instar hoppers (48 h old) of *Hieroglyphus nigrorepletus* were injected with different sub-lethal doses (0·5, 1, 2, 4 and 6 μg/hopper) of triol—and makisterone A, after 3 days the haemocytes of these hoppers were selectively damaged. The granular haemocytes, being the most susceptible cells were completely damaged even by the weakest dose of triol and makisterone A. Prohaemocytes and plasmatocytes were intensely damaged by 1 μg of makisterone A, 2 and 1 μg doses of triol respectively. However, oenocytoids were comparatively resistant cells, but they were also damaged by 2 μg triol and 4 μg makisterone A. Following the emergence of adults from the treated hoppers after 3 days, both granular haemocytes and plasmatocytes were indistinguishable with respect to even weakest dose of both the hormones. Comparatively prohaemocytes were less damaged and could be recognized in adults affected with 1 and 2 μg of makisterone A and triol respectively. However, oenocytoids were completely disintegrated in adults emerged from the hoppers injected with 1 and 2 μg of triol and makisterone A respectively. In adults of both sexes, respective haemocytes developed almost similar pathological condition with regard to each dose.

Keywords. *Hieroglyphus nigrorepletus*; hopper; triol; makisterone A; prohaemocytes; plasmatocytes; granular haemocytes; oenocytoids.

1. Introduction

In a number of insects treated with chemicals including insecticides, the haemocytes were pathologically affected (Feir 1979). However, the effect of application of exogenous ecdysones on the haemocytes, which proved toxic on the growth of certain species, is little known. According to Ohmori and Ontaki (1973), the application of higher concentrations of ecdysterone (a synthetic moulting hormone) on adult fly, *Sarcophaga peregrina* inhibited metabolic activity. Judy and Marks (1971) in *Manduca sexta* (Lepidoptera) applied ecdysterone and recorded the increase in the migratory activity of the blood cells viz. spherule cells and plasmatocytes (in vitro). In *Spodoptera litura*, injection of different doses of β-ecdysone to the larvae of the last stage caused dose based pathological damage to different haemocytes (Nishi 1982). Rao *et al* (1984) investigated the effect of β-ecdysone on the total haemocyte count in late fifth stage, ligated larvae of *S. litura* behind the thorax.

In view of the meagre information regarding the toxicity of the exogenous ecdysones on the haemocytes, a comparative observation was made in the present study, on different haemocytes of *Hieroglyphus nigrorepletus*, following the injection of triol—an analogue of moulting hormone as well as makisterone A—an ecdysone of plant origin on the 5th instar hoppers. *H. nigrorepletus* is a pest of Kharif crops in India. The toxic effect of exogenous ecdysones on insect haemocytes may be important in considering synthetic ecdysoids as one of the tools of insect control.

2. Materials and methods

Both nymphs and adult of *H. nigrorepletus* were collected from the maize crop and adjoining grass lands of the Agricultural farm of the University. They were kept in wooden cages as designed by Hunter-Jones (1956) at $31 \pm 1°C$ and 70–80% RH. Fresh leaves of maize were provided daily.

From this stock newly moulted 5th instar hoppers were removed and kept at the conditions mentioned before. Then 48 h old hoppers were individually injected with either of 0·5, 1, 2, 4 and 6 μg triol or makisterone A in acetone.

Haemocytes were studied in the stained blood smears of treated hoppers after 3 days as well as those of 3 day old adults, both males and females, emerged out of treated hoppers. For this purpose a blood drop was obtained by cutting antenna or coxa-femur joint of the hind leg. The blood smears were stained both in Giemsa's and Leishman's stain for comparison. Further, stained blood smears of untreated or acetone treated hoppers and emerged adults of corresponding age were also prepared as controls. Each treated hopper was used once to obtain blood for smear. After emergence to adult stage it was not possible to take blood drop again from the same individual. Therefore in adult stage blood sample was taken following emergence from similarly treated hoppers of the same stock and age.

3. Results

Four types of haemocytes viz. prohaemocytes (PRs), plasmatocytes (PLs), granular haemocytes (GRs) and oenocytoids (OEs) were recognized both in the nymphal stages as well as adults of *H. nigrorepletus* on the basis of the criteria of Jones (1962) (figures 1–4).

3.1 *Pathological effects in haemocytes* (figures 5 and 6)

3.1a *Five day old 5th instar hoppers*

(i)   *PRs*: Even following the injection of weakest dose (0·5 μg) of both the hormones, the changes followed as eccentric nucleus, broken chromatin, ruptured cell membrane and vacuolated cytoplasm (figures 5a, b; 6a, b). By 1 μg triol, cell membrane was further damaged and cytoplasm became highly vacuolated whereas this dose of makisterone A caused notched appearance of the nucleus and irregular cell membrane. By 2 μg triol these cells became indistinguishable and higher doses further deteriorated the cells. On the other hand, by 2 μg makisterone A, these cells were recognizable but developed many vacuoles in the cytoplasm and nucleus (figure 6d, e). However, stronger doses of makisterone A, also made most of these cells indistinguishable.

(ii)   *PLs*: The effect of weakest dose of triol and makisterone A was evident by ruptured cell membrane, eccentric nucleus and discharge of vacuolated cytoplasm (figures 5a, b; 6a, b). Relatively higher doses of both the hormones caused further damage to these cells and finally only nuclei were left (figures 5c, d; 6d, e).

(iii)   *GRs*: Most of these cells showed ruptured cell membrane and expulsion of granules from the cells even following the injection of 0·5 μg dose of both the
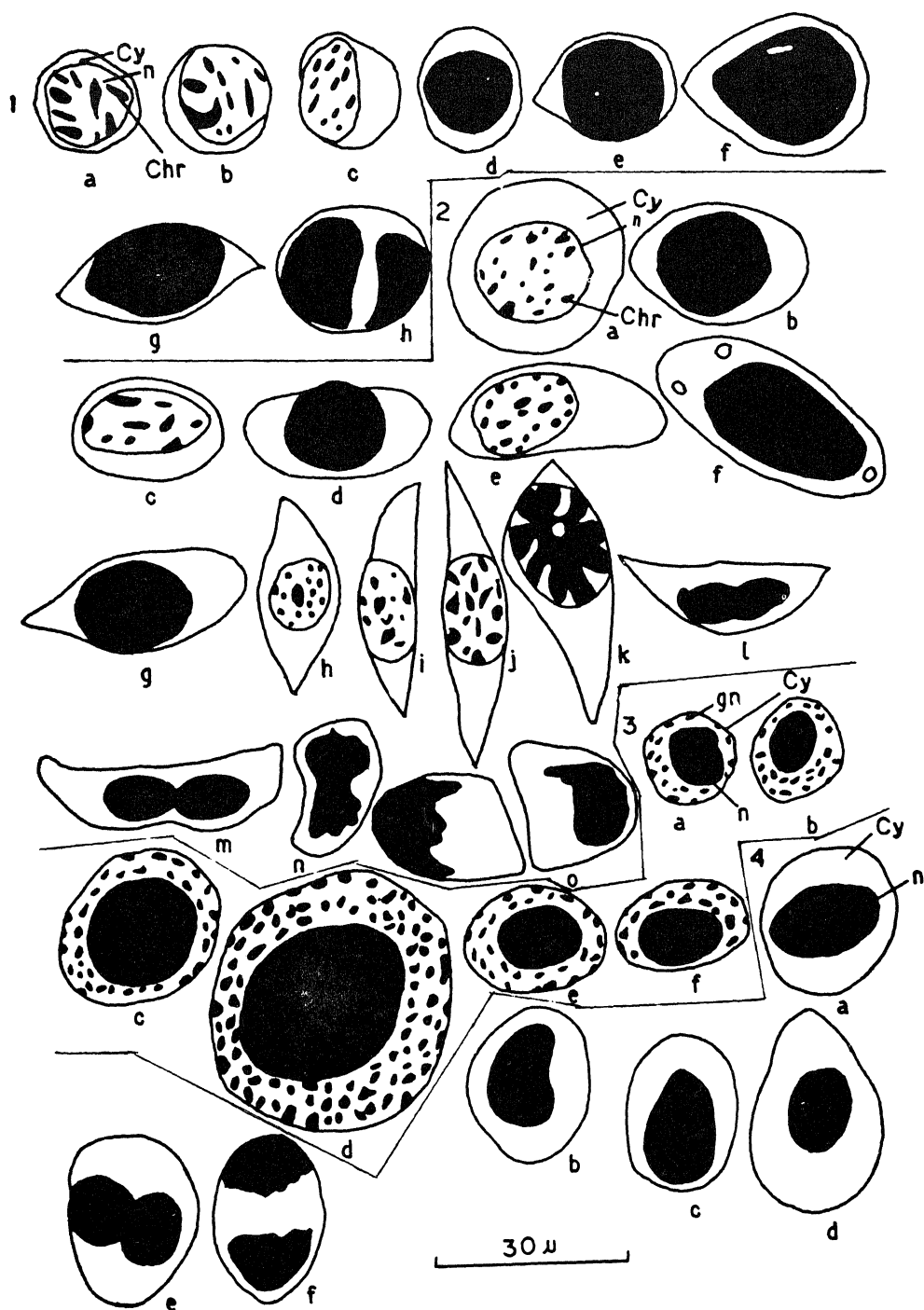
Figures 1–4. Different types of haemocytes of *H. nigrorepletus.* **1.** Prohaemocytes (**a**-**c**, Round; **d**, ovoid; **e and f**, pear shaped; **g**, spindle shaped; **h**, binucleate). **2.** Plasmatocytes (**a**, Round; **b and c**, ovoid; **d**-**f**, elongate; **g**, pear shaped; **h-k**, spindle shaped; **l and m**, bilobed nuclei; **n and o**, stages of mitosis). **3.** Granular haemocytes (**a**-**d**, Round; **e and f**, ovoid); **4.** Oenocytoids (**a**, Round; **b and c**, ovoid; **d**, pear shaped, **e**, binucleate; **f**, late anaphase stage).

Figure 5. Pathological effects in the haemocytes of 5 day old 5th instar hoppers and 3 day old adults of *H. nigrorepletus* following the injection of 0·5 and 2 μg triol/5th instar hopper (48 h old). Enlarged twice from the original magnification (eye piece 10 X, objective oil). **a and b.** Blood smears of affected 5th instar hoppers with 0·5 μg dose, showing ruptured cell membrane of PR, PLs and GR. Granular haemocytes are completely damaged with expulsion of granules. Vacuoles developed in the nucleus of PR whereas only impression of cytoplasm remains in PLs. **c and d.** Blood smears of affected 5th instar hoppers with 2 μg dose, showing only impression of cytoplasm around PLs. **e–g.** Blood smears of affected adults with 0·5 μg dose, showing broken cell membrane in PL, PR and OE. PR shows discharge of cytoplasm and OE shows swollen nucleus and granulated cytoplasm. **h and i.** Blood smears of affected adults with 2 μg dose, showing broken cell membrane in PL, PR and GR. Cytoplasm of PRs and PLs became granulated and shows discharge at places. Whereas GR shows expulsions of granules.
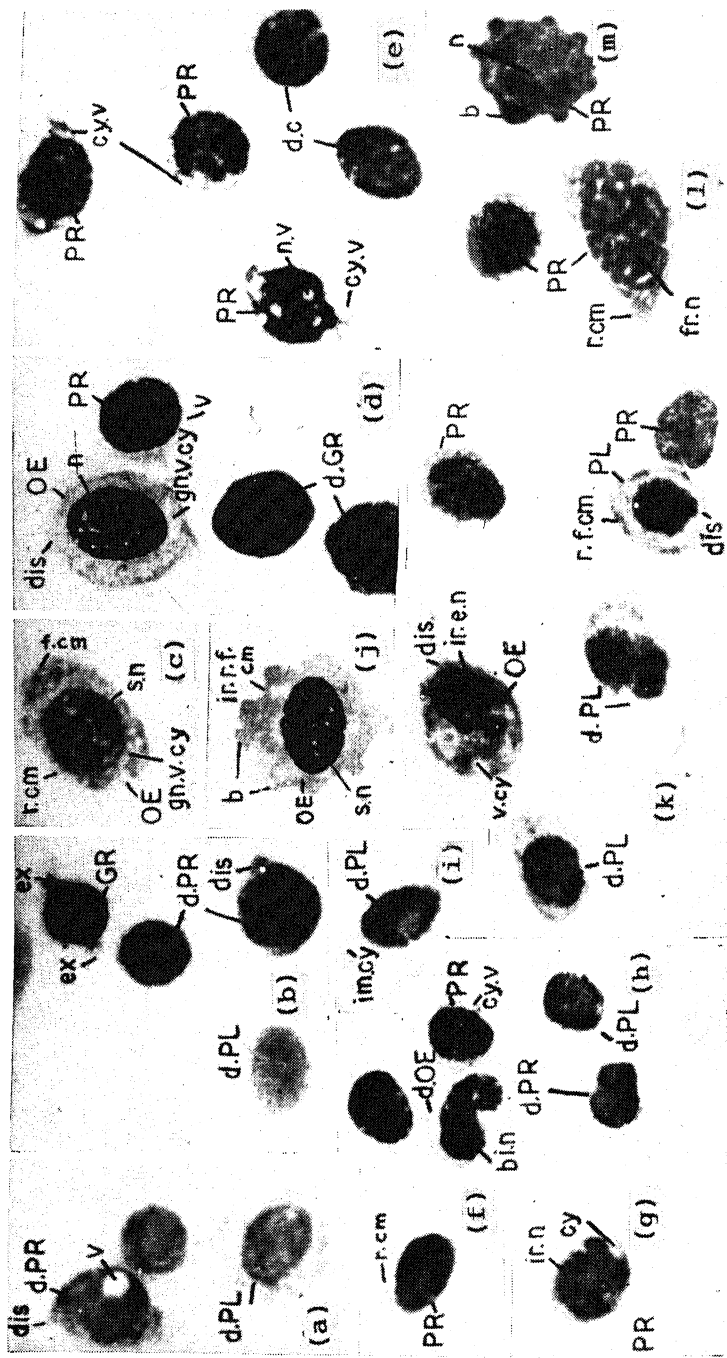
Figure 6. For caption, see p. 208.

hormones (figures 5a, 6b). By 2 μg dose of both the hormones these cells were further damaged but were still recognizable by their remains (figures 5c; 6d).

(iv)   *OEs*: In comparison to other cells, these cells were slightly damaged at 0·5 μg triol and only a few cells showed eccentric position of the nucleus. Whereas by 0·5 μg makisterone A/hopper, the nuclei became irregular and swollen; cell membrane was ruptured at places and cytoplasm became hyaline and vacuolated (figure 6c). The cell membrane became irregular by 1 μg triol whereas by the same dose of makisterone A only nuclei were pushed to eccentric position. Two μg Triol ruptured the cell membrane and cytoplasm was destroyed. Whereas, similar dose of makisterone A developed only hyaline cytoplasm around the swollen nucleus. However, 6 μg of this hormone ruptured the membrane of both cell and nucleus and cytoplasmic discharge.

3.1b   *Haemocytes of 3 day old adult*: The haemocytes of 3 day old adults (both males and females) emerged from the treated 5th instar hoppers (48 h old) were also studied. The important changes are described below:

(i)   *PRs*: The nuclei of these cells showed eccentric position following the injection of 0·5 μg of each hormone (figures 5e, f; 6f-h). By 1 μg of either hormones the membranes of both cell and nucleus became irregular. Further, 2 μg triol caused development of cytoplasmic vacuoles and rupture of the cell membrane. However, similar dose of makisterone A influenced these cells by the appearance of abnormal vacuoles and granules in the cytoplasm as well as fragmented nuclei and bulging in the cell membrane (figures 5i; 6l, m). Relatively higher doses of these hormones caused further deterioration in the cells, which were dose based.

(ii)   *PLs*: As compared to the PRs, these cells were much adversely affected even by 0·5 μg dose of both the hormones, showing ruptured cell membrane and obliteration of the vacuolated cytoplasm (figures 5e; 6h, i). By 2 μg makisterone A, only nuclei

Figure 6.   Pathological effects in the haemocytes of 5 day old hoppers and 3 day old adults of *H. nigrorepletus* following the injection of 0·5 and 2 μg makisterone A/5th instar hopper (48 h old). **a-c.** Blood smears of affected 5th instar hoppers with 0·5 μg dose, showing big vacuole in the nucleus of a PR, broken cell membrane in GR, PRs, PLs and OE with cytoplasmic discharge. GR shows expulsion of granules and OE shows folded cell membrane with swollen nucleus and granulated and vacuolated cytoplasm. **d and e.** Blood smears of affected 5th instar hoppers with 2 μg dose, showing completely damaged GRs and PLs. OE shows granulated and vacuolated cytoplasm ruptured cell membrane with cytoplasmic discharge and swollen nucleus whereas many abnormal vacuoles developed in the cytoplasm and nucleus of PRs. **f-i.** Blood smears of affected adults with 0·5 μg dose, showing ruptured cell membrane of PR, PLs and OE. PR shows irregular nucleus and cytoplasmic vacuoles. There is a bilobed nucleus in OE and only impression of cytoplasm in PLs. **j-m.** Blood smears of affected adults with 2 μg dose, showing broken cell membrane in PRs, PLs and OE. PR shows cytoplasmic bulgings and fragmented nucleus. OE shows highly vacuolated cytoplasm and irregular nucleus, whereas PLs shows folded cell membrane and cytoplasmic discharge.

(*Abbreviations*:   GR, Granular haemocyte; OE, oenocytoid; PL, plasmatocyte; PR, prohaemocyte; b, bulging; bi, bilobed; chr, chromatin material; cy, cytoplasm; d.c, damaged cell; dis, discharge of cytoplasm; e, eccentric; ex, expulsion of granules; fr, fragments; gn, granule; im, impression; ir, irregular; n, nucleus; r.f.cm, ruptured and folded cell membrane; s, swollen; v, vacuole).

were left. Whereas similar doses of triol caused cytoplasmic discharge and development of projections in the nuclei (figures 5h, i; 6k, l).

(iii) *GRs*: Being the most sensitive cells in the smears, these cells became indistinguishable even by the application of the lowest dose of both the hormones.

(iv) *OEs*: These cells were comparatively less affected than other cells by the injection of 0·5 μg triol. Their nuclei were eccentric and swollen; chromatin material became thick and compact; cytoplasm developed abnormal granules (figure 5 g). Whereas by 0·5 μg makisterone A nucleus became swollen, often bilobed but cytoplasm was hyaline (figure 6 h). By 1 μg triol cell membrane became irregular and ruptured showing some flow of cytoplasm. Makisterone A caused such changes following the injection of 2 μg dose (figure 6j, k). Maximum effect was produced by 6 μg triol which destroyed most of the cells than by similar dose of makisterone A.

4. Discussion

From the present data it is concluded that injection of different sub-lethal doses of triol and makisterone A to 5th instar nymphs of *H. nigroreplelus* caused development of pathological symptoms in different types of haemocytes, which were dose based. Generally changes in the cells followed the sequence of pushing of nuclei to eccentric position, abnormal vacuolization in both cytoplasm and nucleus, cytoplasmic projections and bulging, irregular shape of the cell, ruptured cell membrane, intense vacuolization of cytoplasm, cytoplasmic discharge, fragmentation as well as disintegration of the nucleus and finally loss of cellular identity. The most sensitive cells to these hormones were granular haemocytes whereas plasmatocytes, pro-haemocytes and oenocytoids were comparatively susceptible in descending order. Thus the oenocytoids were the most resistant cells, probably due to their thicker nature in comparison to other types of cells. Triol is more effective to destroy these cells as compared to makisterone A.

It is possible that these hormones when injected to the haemocoel also diffused into the haemocytes and became toxic to them like the insecticides. The pathological effects of exogenous hormones on the haemocytes of insects was earlier recorded in only *Spodoptera litura* (Nishi 1982; Rao *et al* 1984) and in *Galleria mellonella* and *Tenebrio molitor* (Farks 1984). Therefore the present information on *H. nigroreplelus* is new among Orthopterous insects. It is interesting to record that the pathological effect of exogenous hormones on the haemocytes of the above mentioned species are comparable to that of insecticides in other species e.g. *Calliptamus italicus* (Tareeva and Nenyukov 1931), *Dysdercus cingulatus* (Zaidi and Khan 1977), *Rhopalosiphum maidis* (Behura and Dash 1978) and a review by Feir (1979).

Although application of both moulting hormones and insecticides' cause similar types of pathological conditions and eventually destruction of these haemocytes (which is the important site of intermediary metabolism), control of insects by the analogues of the ecdysone (moulting hormone) may be preferred because these are definitely non-toxic to crops, live-stock and human beings.

Acknowledgements

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Bursal glands of Reduviidae (Insecta—Heteroptera)

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Abstract. In Reduviidae, the glandular outgrowths from the bursa either as paired or as a single median gland distinctly different from the paired subrectal glands are described as the bursal glands. The occurrence of such female accessory glands has been investigated in 22 species of Reduviidae. Based on their development, the glands are classified into 3 types. The histomorphology of the gland in various species is given.

Keywords. Reduviidae; bursal gland; spumaline.

1. Introduction

The bursal gland found in most of the reduviids are known to secrete spumaline. This glandular structure from the bursa had been reported by many earlier workers as cement gland (Galliard 1935; Beament 1949; Dupuis 1955; Louis and Kumar 1973), spermatheca (Larsen 1938; Davis 1955; Wygodzinsky 1966; Cobben and Wygodzinsky 1975) and Spermathecal gland (Scudder 1959). In the present study the glandular structure associated with the female reproductive tract secreting spumaline has been considered as accessory glands.

2. Materials and methods

Female reproductive system of newly emerged adults and egg laying females were dissected out in 8% insect Ringer's solution and fixed in warm bouins fluid for 12 h. After repeated washing in 70% alcohol these were preserved in 75% alcohol for study. Drawings were made using a camera lucida. For histological studies, materials were dehydrated and after following Peterfiz celloidin-double embedding process, sectioned at 5–6 μm thickness. The sections were stained with Ehrlich's iron haematoxylin-eosin and mounted in DPX.

3. Results

3.1 Gross morphology

The bursal gland has been found to have undergone remarkable variation in its morphology and on this basis it is distinctly divided into 3 types.

3.1a *Vermiform type (Type-I)*: This type is found in *Lophocephala guerini* Lap. (Harpactorinae) (figures 1B, 6K), *Diaditus errabundus* Dist. (Stenopodinae) (figures 2A, 6D), *Apechita mesophyrrha* Reut. (Acanthaspidinae) (figure 3B) and *Lisarda annulosa* Stal (figure 3A). In these insects the gland is vermiform and is glandular throughout

its length without regional differentiation. It is comparatively shorter in all the above species except in *L. guerini* where it is found to be much elongated (figure 1B). The terminal region of the gland appears swollen in *A. mesopyrrha* (figure 3B).

3.1b *Basal ampulliform type (Type-II)*: This type is found in Stenopodinae, Emesinae, Ectrichodiinae and Salyavatinae. Here the gland can be distinctly differentiated into 3 regions, a basal short muscular duct followed by a small ampulliform glandular region and a terminal vermiform glandular region. In *Sastrapada baerensprungi* Stal. the distal half of the vermiform glandular region is granulated and light pink coloured. The dark proximal half is bulged. There is a small constriction between the bulged secretory region and the ampulla (figures 2D, 6I). In *Bardesanes signatus* Dist. a part of the vermiform region, immediately following the ampulla is prominently granular (figure 2C). The bursal gland of *Oncocephalus modestus* Reut. (figures 2B, 6H) can be considered as a typical example of the basal ampulliform type. In *Petalochirus indicus* Reut., the basal region is swollen as the glandular segment (figures 3C, 6A). It should be noticed that the paired Spermathecae, each with 4 rami are distinctly different from the median accessory gland (figure 6E). In *Ghilianella* sp.n. of the sub family Emesinae, the ring gland (figure 1C) on the dorsal wall of the bursa, encircles the bursal gland. Here, the basal ampulla is elongately bulbous and the apical opaque vermiform segment remains highly convoluted and coiled (figure 6B, C). The bursal gland of *Haematorrhophus nigroviolaceus* Reut. is very long and lies coiled over the bursa. The vermiform segment is transparent so that the chitin lined lumen is visible. The glandular ampulla is oblong in shape (figures 1D, 6J).
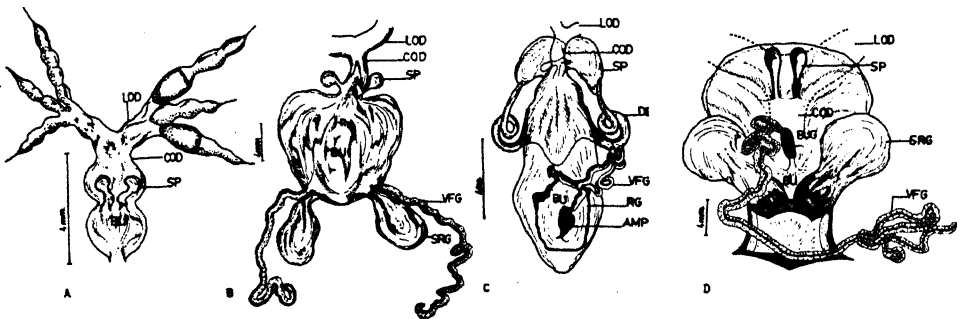
Figure 1. Absence of bursal gland in **(A)** *Holoptilus melanospilus*, **(B)** *Lophocephala guerini*, **(C)** *Ghilianella* sp. and **(D)** *Haematorrhophus nigroviolaceus*.
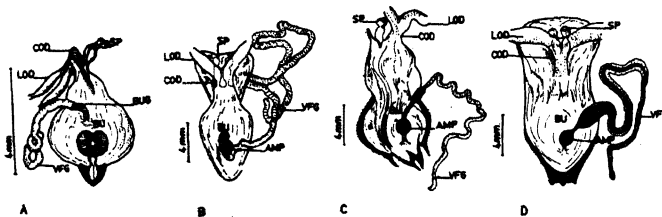
Figure 2. Bursal gland of **(A)** *Diaditus errabundus*, **(B)** *Oncocephalus modestus*, **(C)** *Bardesanes signatus* and **(D)** *Sastrapada baerensprungi*.
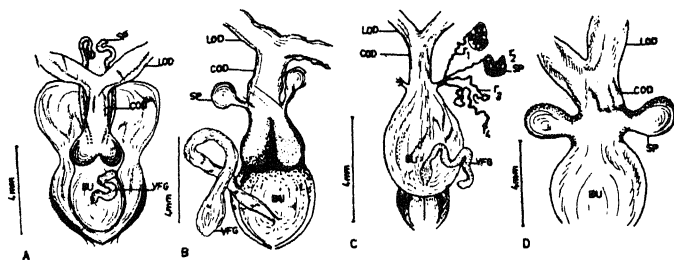
Figure 3. Bursal gland of (A) *Lisarda annulosa*, (B) *Apechita mesopyrrha*, (C) *Petalochirus indicus* and (D) Absence of gland in *Acanthaspis siva*.

3.1c *Distal ampulliform type (Type-III)*: It is characteristically found in Acanthaspidinae and Piratinae. In this type, the sole secretory part is the terminal bulb that varies in shape. It is followed by a long highly muscular conducting duct. The entire length of the duct has chitinous spiral lining.

In *Pirates affinis*, Serv. the glandular bulb is somewhat elliptical and appears incompletely segmented (figures 4D, 7F). Whereas in *Ectomocoris tibialis* Dist. and *Ectomocoris vishnu* Dist. the gland is distinctly pear shaped (figures 5C, 6G). The distal half of the conducting duct in *E. gangeticus* Bergr. and *E. tibialis* Dist. is more muscular than the proximal half (figures 5B, D, 6F). In *Ectomocoris atrox* Stal. the gland is club shaped. Here the gland along with a part of its duct remains enveloped in a membraneous sheath (figure 5E). In *Catamiarus brevipennis* Serv. there is a small prominence at the junction of the gland and the duct (figure 5A). The apical globular gland appears bilobed in *Acanthaspis pedestris* Stal. (figure 4B) whereas in *Acanthaspis quinquespinosa* Fabr. it is kidney shaped (figure 4C). Any type of bursal gland is found absent in *A. siva* Dist. (figure 3D), *Edocla slateri* Dist. (figure 4A) and *Platymeris laevicollis* Dist. The same condition is also observed in *Holoptilusmelanospilus* Walk. which lacks accessory glands (figure 1A).

The eggs of *L. guerini* are found firmly glued to each other and to the substratum using the dark spumaline secreted by the bursal gland. The eggs of other reduviid species are deposited either loosely and scatterdly. In Emesinae and Stenopodinae the spumaline is deposited in the form of longitudinal bands or dots and the eggs are partially glued to the substratum. The eggs of stenopodinae are also found covered with a thin smear of brownish black spumaline to which sand and soil adheres. Most of the piratines bury their eggs in the soil with an accumulation of black gluing material at the caudal end, but exposing the operculum and collar filaments. None of the eggs of Acanthaspidinae and Salyavatinae is glued to the substratum though a certain amount of spumaline is found smeared around the eggs of *P. indicus*.

3.2 *Histomorphology*

The histomorphology of the bursal glands, so far studied has been found to be uniformly similar in all the species of the sub families Harpactorinae, Salyavatinae, Ectrichodiinae, Stenopodinae, Acanthaspidinae and Piratinae.

In general, the glandular sector of the bursal gland is composed of numerous small globular secretory units, in the acinous pattern (figures 6L, M, N; 7A, C, E, F, K, L; 8E). Each globular secretory cell unit has chitinous boundary and all the units are
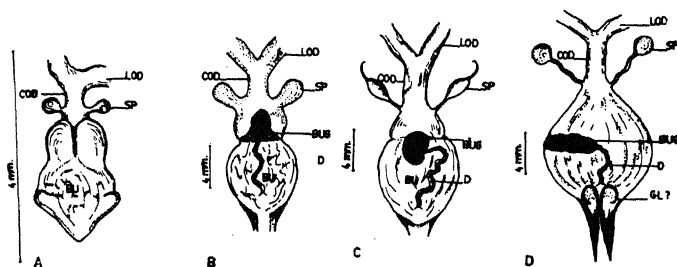
Figure 4. Absence of bursal gland in (A) *Edocla slateri*, (B) *Acanthaspis pedestris*, (C) *Acanthaspis quinquespinosa* and (D) *Pirates affinis*.
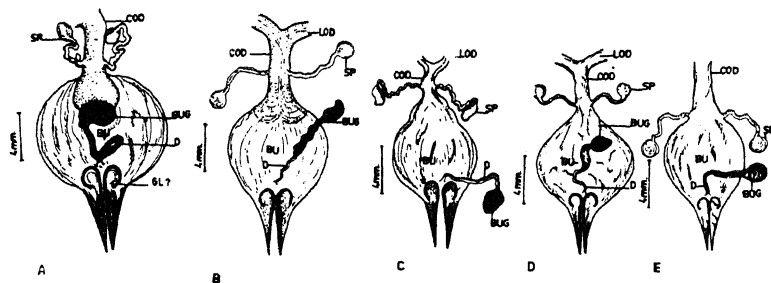
Figure 5. Bursal gland of (A) *Catamiarus brevipennis*, (B) *Ectomocoris gangeticus*, (C) *Ectomocoris vishnu*, (D) *Ectomocoris tibialis* and (E) *Ectomocoris atrox*.

connected to the central collecting chamber by minute canaliculi. The central collecting chamber is also lined with chitinous intima bearing minute pores of the canaliculi (figures 7D, M; 8K). The muscle fibres around the gland are feeble and the basement membrane is not well defined in sections (figures 6N; 7B, M; 8H, I).

The conducting duct from the gland to the bursa is highly muscular. The inner lining of intima forms transverse folds throughout the lumen and in longitudinal sections such folds appear as structs projecting into the lumen (figures 7H, J; 8B, C).

A comparative study of the sections of different bursal gland in various species reveals that though all have similar acinous type of secretory components, certain degree of specialization has been achieved in different species. In *L. guerini*, a layer of cuboidal cells with secretion bordering the intima is followed by an aggregation of numerous secretory goblet cells (figure 6N). At the peak of their secretory activity most of these cells are filled with a dense material and their nuclei are found to be disintegrated. In some cells they remain as darkly stained round bodies. Some of the cells are found empty after having their secretions transferred through the canaliculi into the central chamber. Therefore, the secretory activity resembles the holocrine type.

In the case of *P. indicus* the gland appears to have an outer cortical region composed of compactly arranged cells, with distinct nuclei and a medullary region composed mostly of highly vacuolated cells and a few cells with almost disintegrated nuclei (figure 7A, B). The duct has chitin lined lumen and thick circular muscles. The oblong basal segment is the main source of spumaline in *H. nigroviolaceus*. Here, the secretory cells are found to be more compactly arranged and active in the basal
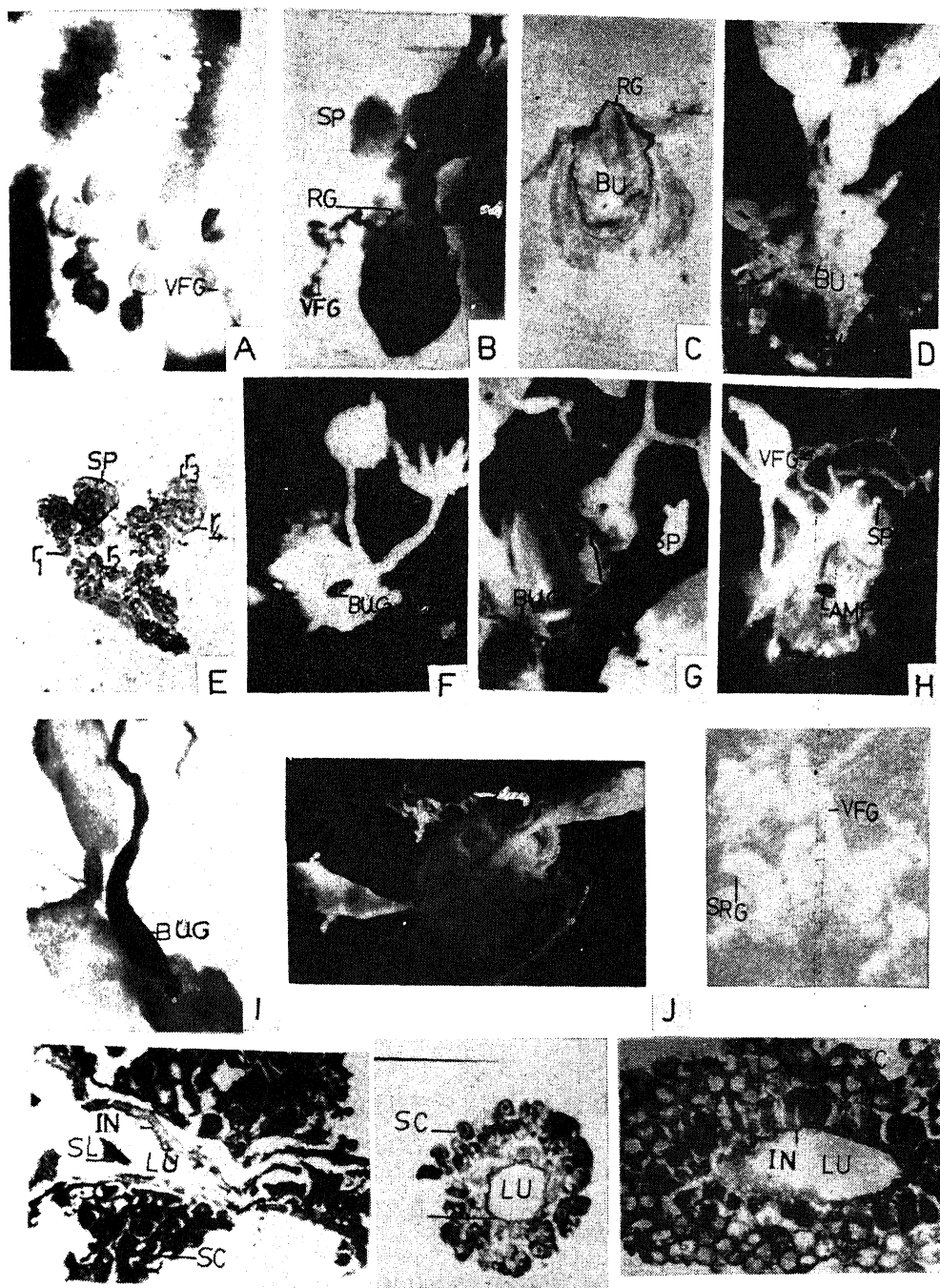
Figure 6. For caption, see next page.

segment than at the apical vermiform segment (figure 6M). Their nuclei appear to have undergone fragmentation during the secretory phase. The short duct with strong circular muscle conduct the spumaline. Histologically, *O. modestus* has similar conglomeration of glandular cells in the epithelium of the basal ampulla (figure 6L). Cells of the ampulla and the vermiform region are with quite large nuclei and the dark viscous secretion of each cell is later emptied into the chitinous central channel. The duct of the gland in these species is relatively short. The duct is characterised by strong circular and longitudinal muscles which regulate and release the secretory products into the bursa.

The apical glandular segment of the bursal glands of Acanthaspidinae (*A. pedestris*, figure 7E and *A. quinquespinosa*, figure 7C) and Piratinae (*P. affinis*, figure 7F and *E. vishnu*, figure 7K). *E. tibialis* (figure 7L) and *C. brevipennis* (figure 8E) have almost similar histomorphology and secretory activity. The gland appears to have an outer cortical layer consisting of compactly arranged goblet cells and a medullary region consisting of both vacuolated and secretion filled cells, distinctly demarcating it off from the cortex. The transformation and migration of cortical goblet cells towards the medullary region and the discharge of secretion into the lumen during the secretory phase could be clearly observed in *C. brevipennis*. The central lumen lined by intima, is in continuation with that of the conducting duct and the junction is constricted (figures 7F, H).

In the newly emerged adult of *C. brevipennis*, the gland is relatively small. A group of cells, confined to the periphery of the gland, border the chitin lined lumen which is quite spaceous (figure 8D, H). Gradually, proliferation of these cells occurs towards the centre and the central lumen become narrow by the accumulation of cells (figure 8E, I). During the secretory phase, the nuclei enlarge and actively participate in the synthesis of spumaline (figure 8I). At the peak of secretory activity, the nucleus of each cell divides into two to four fragments and later they disintegrate (figure 8F, J, L). After the discharge of secretory product into the chitinous canaliculi, the cells are found to have been almost obliterated, suggestive of holocrine type of secretion (figure 8G, K).

In the case of *E. tibialis* the lumen contains a coarse granular secretion (figure 7M), whereas in *A. pedestris* (figure 7D), *A. quinquespinosa* (figure 7C), *C. brevipennis* (figure 8K) and *P. affinis* (figure 7G) the secretion is finely granular and remains more condensed in the form of a dark viscous fluid.

The conducting duct is highly convoluted and the muscular wall consists of an outer strong striated longitudinal muscle layer and an inner circular muscle layer (figures 7I, J; 8C, M). The intima is relatively thick, lining the inner wall of the duct and is produced into wrinkled lobes as in *P. affinis* (figure 7F, H, I). The proximal

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Figure 6. Internal female reproductive organs of Reduviidae showing bursal glands. **A.** *Petalochirus indicus* (5 × 10x). **B.** *Ghilianella* sp. (20 × 10x). **C.** Ring gland on the bursal wall of *Ghilianella* sp. (40 × 10x). **D.** *Diaditus errabundus* (20 × 10x). **E.** *Spermatheca* of *Petalochirus indicus* (40 × 10x). **F.** *Ectomocoris tibialis* (5 × 10x). **G.** *Ectomocoris vishnu* (20 × 10x). **H.** *Oncocephalus modestus* (5 × 10x). **I.** *Sastrapada baerensprungi* (20 × 10x). **J.** *Haematorrhophus nigroviolaceus* (5 × 10x). **K.** *Lophocephala guerini* (20 × 10x). **L.** Section through the basal glandular ampulla of *O. modestus* (40 × 10x). **M.** TS through the basal glandular ampulla of *H. nigroviolaceus* (40 × 10x). **N.** TS of the vermiform gland in *L. guerini* (40 × 10x).
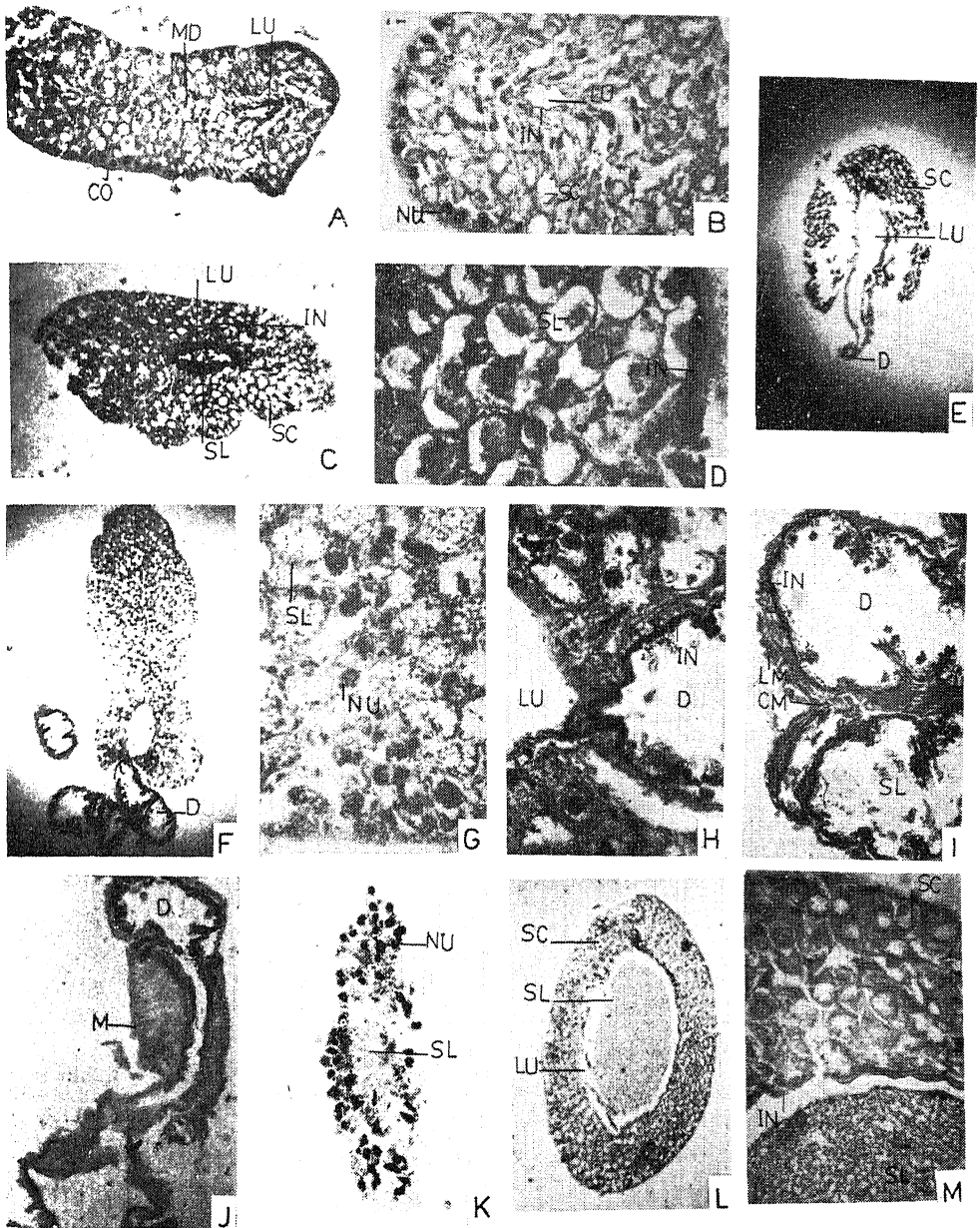
Figure 7. **A.** LS of the bursal gland in *Petalochirus indicus* showing cortical and medullary differentiation of epithelium (20 × 10x). **B.** Magnified view of the same (40 × 10x). **C.** LS of the terminal glandular ampulla in *Acanthaspis quinquespinosa* (20 × 10x). **D.** Enlarged view of the bursal gland in *A. pedestris* showing the cells carrying coarse secretion (40 × 10x). **E.** LS of the same gland and duct (5 × 10x). **F.** Section of the gland and the duct in *Pirates affinis* (20 × 10x). **G.** Magnified view of the same segment (40 × 10x). **H.** LS through the junction of the gland and the duct (40 × 10x). **I.** Enlarged view of the duct showing the thick musculature and intimal lining (40 × 10x). **J.** LS of the duct opening into the bursa (20 × 10x). **K.** LS of the bursal gland in *Ectomocoris vishnu* (20 × 10x). **L.** TS through the bursal gland of *E. tibialis* (10 × 10x). **M.** Enlarged view of the same showing secretory area and intimal lined lumen filled with spumaline (40 × 10x).
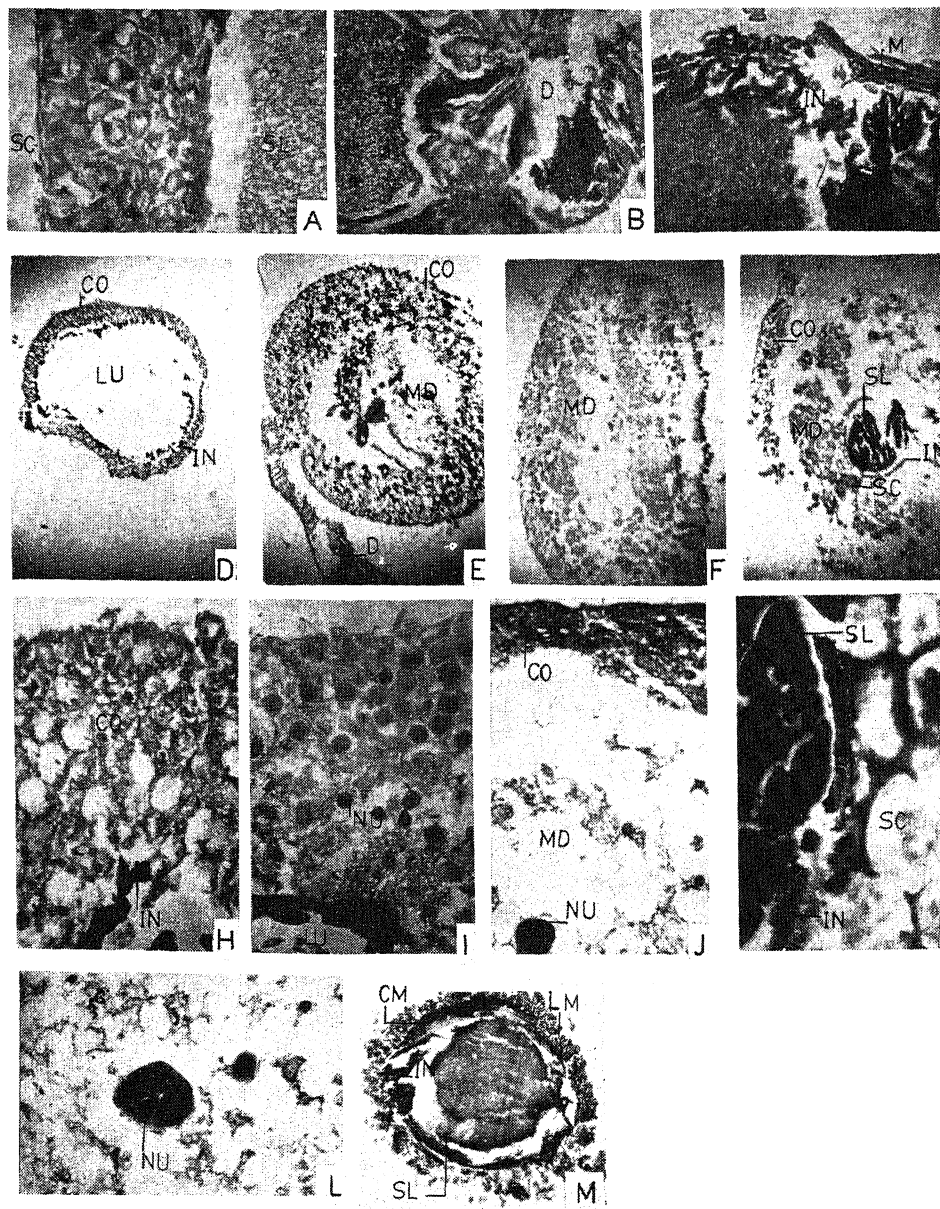
Figure 8.

region of the duct is invested with powerful muscle (figure 7J) that serve to pump the viscous material with certain force into the bursa at the time of oviposition. The tortous course of the duct and the corrugated intimal lining of the lumen necessitates strong muscular action for pumping the viscous spumaline and so the duct is invested with thick muscle layers.

4. Discussion

A single median accessory gland distinctly arising on the bursa which is entirely different from the subrectal glands as found in most species of Reduviidae, other than Harpactorinae has been therefore designated here as the bursal gland. Occurrence of such a gland in *Rhodnius* and *Triatoma* was reported earlier by Galliard (1935) and he has described it as a tubular accessory gland, opening dorsally into the genital chamber, secreting material to cement the eggs to the substratum.

The status of spermatheca in Heteroptera began to be questioned when Larsen (1938) pointed out structural similarities between the bursal gland (in terresterial Heteroptera) and the spermatheca in several species of aquatic Heteroptera. Davis (1955) subsequently made an attempt to homologise the median accessory gland (bursal gland) of Reduviidae to median spermatheca of Miridae. He further went to the extent of considering this gland in Reduviidae as their spermatheca and the presence of muscles on its tubular part was emphasised as an evidence in favour of his assertion. Pendergrast (1957) confirmed the occurrence of a single accessory gland in *Rhodnius*. Scudder (1959) has found great variations in the structure of median accessory glands in Reduviids. Dupuis (1955) suggested that the receptaculum semenis has changed its function to serve as accessory gland in Reduviidae. In his monograph on Emesinae, Wygodzinsky (1966) has given a detailed account of female accessory gland and other ectodermal structures of the female reproductive system. In his monograph, a vermiform gland of *Emesaya brevipennis* has been described as having 3 distinct regions namely: a short membraneous ductus recepta-

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Figure 8. A–C. *Ectomocoris tibialis*. A. Section of the gland showing the active secretory phase and disintegration of nuclei (40 × 10x). B. LS at the beginning of the duct from the gland (40 × 10x). C. Section showing the musculature and intimal lining of the duct (40 × 10x). D–M. *Catamiarus brevipennis*. D. TS through the bursal gland of newly emerged adult (10 × 10x). E. LS of the gland showing cortical and medullary region as well as the muscular duct (10 × 10x). F. Enlarged view of the gland in the final stage of secretory phase (10 × 10x). G. Section of the gland showing the lumen containing spumaline (10 × 10x). H. Enlarged view of the section D showing non secretory phase (40 × 10x). I. Enlarged view of the section E showing dense nuclei in active phase of secretion (40 × 10x). J and L. Enlarged view of the gland at scenescence with aggregation of 2 or 4 nuclei (40 × 10x). K. Magnified view of section G, showing the empty cells and the chitin lined central lumen containing dark secretion (40 × 10x). M. TS of the duct showing thick musculature and spumaline in the lumen (40 × 10x).

(Abbreviations: AMP, Ampulla; BU, bursa; BUG, bursal gland; CO, cortex; COD, common oviduct; D, duct; DI, diverticula; GL, gland; IN, intima; LM, longitudinal muscle; LOD, lateral oviduct; LU, lumen; M, muscle; CM, circular muscle; MD, medulla; NU, nucleus; RG, ring gland; r_1, r_2, r_3, r_4, rami; SC, secretory cell; SL, spumaline; SP, spermatheca; SRG, subrectal gland; VFG, vermiform gland).

culum which is inserted on the dorsal wall of the vagina followed by a pyriform capsula seminalis upon which is inserted a convoluted irregularly shaped glandula apicalis having a very narrow lumen. Later, Cobben and Wygodzinsky (1975) reported in the case of 4 species of Emesinae similar structures as vermiform glands. An accessory gland resembling the vermiform gland of Emesinae has been reported also in the Stenopodine species such as *Stenopoda wygodzinsky* and *Narvesas carolinensis* but it has been reported to be absent in another stenopodine species *Oncocephalus antipodus* (Cobben and Wygodzinsky 1975). They also considered these structures (vermiform giand) as true spermatheca, supporting the suggestion made earlier by Davis (1955). Matsuda (1976) has also described the median accessory glands in Heteroptera as the vermiform spermatheca. All the 4 stenopodinae species presently studied, including *Oncocephalus modestus*, have well developed bursal gland (figure 2A-D).

Louis and Kumar (1973) have reported a single accessory gland (similar to bursal gland type I) in a tribelocephaline species and in a stenopodine species a very elongated and highly developed accessory gland (similar to bursal gland type II). But they did not recognise any accessory glands in Ectrichodiinae, Salyavatinae and Emesinae.

Occurrence of ring gland on the wall of the genital sac has been traced in 3 *Ghiliallelia* species by Cobben and Wygodzinsky (1975). In the present study a *Ghilianella* species has been found to have both the vermiform gland and the ring gland. But the glandular nature of the so called ring gland could not be assessed though it is found pigmented and does not suggest any secretory function. However, the present investigation positively supports the report of Galliard (1935) and under no circumstances the bursal gland described here contain spermatozoa.

It is an interesting fact that both pairs of subrectal glands and bursal glands are found only in the harpactorine species, *L. guerini.* The existence of these two pairs of glands is a primitive character and suggests the divergence of this species from its parental harpactoroid stock having only the subrectal glands. Thus the bursal gland appears to have its origin as paired vermiform gland, arising at the posterior region of the bursa, in close proximity of the subrectal gland.

It could be presumed that as the vermiform bursal gland has shifted its position more anteriorly from the proximity of the subrectal glands towards the mid dorsal position on the wall of the bursa. A comparative analysis of the morphology and development of the bursal gland in reduviids in the present investigation also reveals that they have undergone various degrees of alteration in size, shape and regionation. Thus the vermiform gland in the course of development, has acquired a basal ampulla and a short muscular duct. Such a bursal ampullate type of bursal gland has been recorded in Ectrichodiinae, Stenopodinae, Emesinae and Salyavatinae, a condition reported earlier by Wygodzinsky (1966) and Cobben and Wygodzinsky (1975) in Emessinae and Stenopodinae. The occurrence of vermiform gland in some species of Stenopodinae, Salyavatinae and Acanthaspidinae can be considered as their probable phylogenetic relationship with the primitive forms. The terminal ampullate type found only in Acanthaspidine and Piratine species have been considered here as highly specialized, next to the basal ampullate type. The total absence of the gland in a few Acanthaspidinae species like *A. siva, E. slateri* and *P. laevicollis* is considered as a secondary specialization.

A comparison of the histology of the vermiform gland of *L. guerini* with that of the bursal gland in all the other species investigated confers that they are all basically

similar, irrespective of the subfamily to which they belong. The vermiform type is glandular throughout its length as found in the section of *L. guerini* whereas in *H. nigrovio-laceus* and *O. modestus* the basal ampulla is the main secretory region. A gradual disappearance or loss of the vermiform segment may have caused the transformation of the gland towards the development of a terminal ampullate type.

Lococo and Huebner (1980a, b) have made contributions to the understanding of the development and ultra structure of the accessory gland in *R. prolixus*. The gland with a distal synthetic region and a proximal muscular duct belongs to the terminal ampullate type of gland found in Acanthaspidinae and Piratinae. The structural details of such glands given in the present investigation does not vary much from the account given by these authors on the ultrastructure of the cement gland. They have described that the synthetic region is composed of numerous secretory units. Proteinaceous secretion from these synthesizing sites passes through cuticular lattice-like end-apparatus and then gets out through ducts to the central chitinous duct. The secretory cells have septate junctions with the cuticular ductules. The ductules, and the central chamber are in continuation with the proximal muscular duct. The latter has corrugated circular lining, a thin epithelium rich in microtubules and thick striated longitudinal muscles. On the contrary, it is observed in *P. affinis*, *C. brevipennis* etc that the conducting duct has an inner glandular epithelial layer followed by strong well developed circular muscles and outer thin longitudinal muscles. The lumen has thick chitinous lining which is produced as corrugated lobes into the lumen.

The bursal glands by virtue of their histomorphological identity shows their clear distinction from subrectal glands.

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Subrectal glands of Reduviidae (Insecta—Heteroptera)

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Abstract. The subrectal glands associated with the female reproductive system is a characteristic feature of Harpactorinae. These glands produce spumaline for gluing eggs to the substratum in a mass. Occurrence of subrectal glands have been investigated in 18 reduviid species. The gross morphology and histomorphology of these glands are illustrated and described.

1. Introduction

The subrectal glands of Reduviidae are concerned with secretion of spumaline. These paired ectodermal glands are opening either jointly or independently at a posterior region of the bursa either in close proximity or into the genital pore. A general description on the occurrence and morphology of these glands have been contributed by Kershaw (1909), Readio (1927), Miller (1956), Scudder (1959), Davis (1969), Louis and Kumar (1973), Swadner and Yonke (1973a, b) and Cobben and Wygodzinsky (1975). Anatomical and histological details of subrectal glands were given by Barth (1961). In the present investigation, the structure and histomorphology of subrectal glands have been carried out to assess them as colleterial glands.

2. Materials and methods

Female reproductive system of newly emerged adults and egg laying females were dissected out in 8% insect Ringer's solution and fixed in warm bouins fluid for 12 h. After repeated washing in 70% alcohol, these were preserved in 75% alcohol for study. Drawings were made using a camera lucida. For histological studies, materials were dehydrated and after following Peterfiz celloidin-double embedding process, sectioned at 5–6 μm thickness. The sections were stained with Ehrlich's iron haematoxylin-eosin and mounted in DPX.

3. Results

3.1 *Gross morphology*

The subrectal glands have been found to be of the vesicular type and the extent of their development· varies from species to species. Generally, harpactorines of the tropical rain forests and scrub jungle ecosystem (e.g. *Sycanus versicolor* Dohrn, *Rhinocoris marginatus* Fabr, *Rhinocoris fuscipes* Fabr, *Rhinocoris kumarii* sp.n.) have relatively large (5–10 mm) subrectal glands and this is correlated with their habit of

gluing their eggs in compact masses to the substratum by using large quantum of spumaline secreted by them. The subrectal glands found in *Neohaematorophus therasii* sp.n., *Coranus* spp., *Euagoras plagiatus* Stal. and *Coranus obscurus* Stal. are small (1–3 mm) in proportion to the size of the insect. They secrefe little spumaline and deposit eggs in loose batches.

The size, shape and colour of the subrectal glands change depending on the secretory activity of the gland. During the time of oviposition, when this accessory gland is at the peak of its secretory phase, it is found distended in the form of a massive sac and appears dark. During the non ovipositional period it is found to be light yellowish, shrunken and collapsed.

The subrectal glands of *S. versicolor* are paired elongate and convoluted, arising as dissimilar pouches from the postero-dorsal region of the genital chamber (figure 2A). The common opening of the two unequal lobes lies close to the genital pore. In *R. marginatus* they are elliptical and dark when filled with the secretion (figure 2B) but are found to be transparent and collapsed when non active as in the newly emerged adults. There is also a pair of distinct diverticula arising near its base. In *R. kumarii* the glands are long and globular near their apices (figures 2D, 6B) and in *R. fuscipes* they are rather short and sac-like with two diverticula from the anterior margin (figures 2C, 6A). In *Platerus pilcheri* Dist. also the subrectal glands appear as two long lobes having a common opening (figure 3A).

In *N. therasii* sp.n; (figure 3B) *Harpactor* species (figure 3C), *C. obscurus* (figure 4A), *E. plagiatus* (figure 5A), *Coranus spiniscutis* Reut. (figure 3D), *Coranus wolffi* Leth (figure 4C), *Coranus atricapillus* Dist. (figure 4D) and in another *Coranus* species (figure 4B), the subrectal glands remain separate, each opening independently either into the genital chamber or into the genital opening and at times it is difficult to discern the exact position of their opening.

Among the several harpactorines examined, *Rhaphidosoma atkinsoni* Bergr. (figure 5B), *Irantha armipes* Stal. (figure 5C) and *Polydidus armatissimus* Stal. (figure 5D) have been found lacking subrectal glands. Therefore their source of gluing material may be ovidual in origin as in several other Heteroptera.

Lophocephala guerini Lap. alone has been found to possess both the subrectal glands and bursal glands, in pairs, opening in close proximity of each other on either side, at the posterior extremity of the bursa (figures 1A, 6C). Here, the subrectal glands are saccular, whereas the bursal glands are very long and vermiform. In the case of *Haematorrhophus nigroviolaceus* Reut. (Ectrichodiinae) (figures 1B, 6D), apart
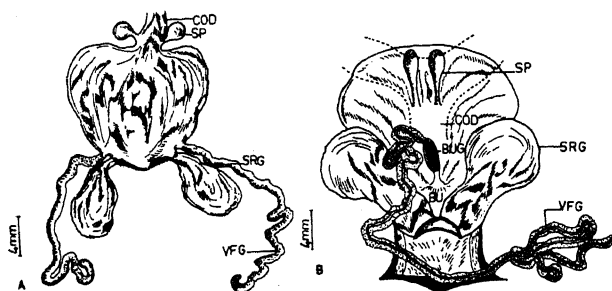
Figure 1.   A. *L. guerini.*   **B.** *H. nigroviolaceus.*
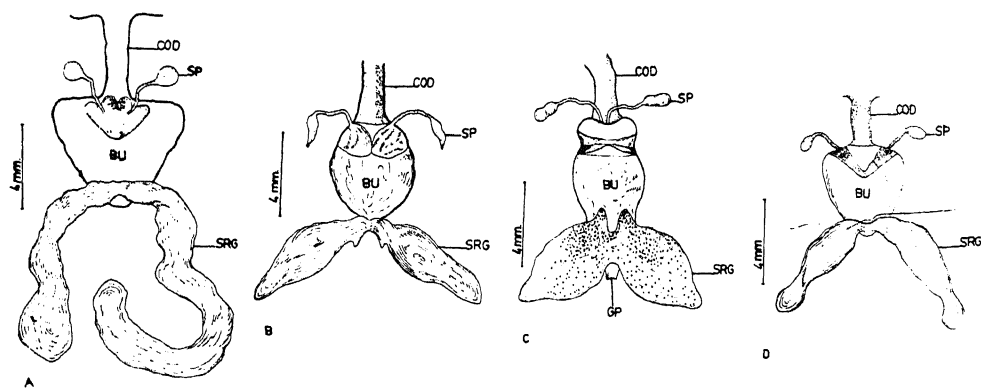
Figure 2. **A.** *S. versicolor.* **B.** *R. marginatus.* **C.** *R. fuscipes.* **D.** *R. kumarii.*
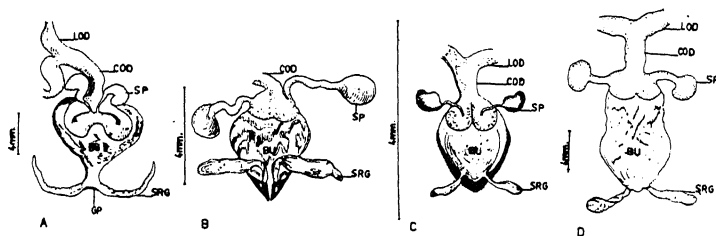
Figure 3. **A.** *P. pilcheri.* **B.** *N. therasii.* **C.** *Harpactor* sp.n. **D.** *C. spiniscutis.*
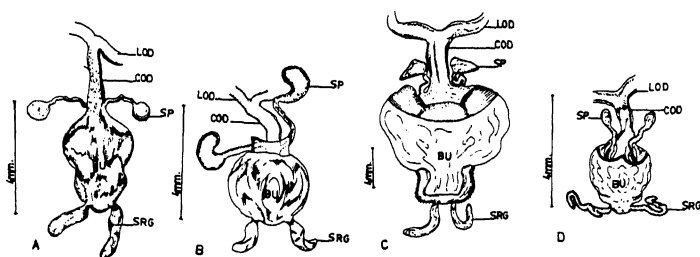
Figure 4. **A.** *C. obscurus.* **B.** *Coranus* sp.n. **C.** *C. wolffi.* **D.** *C. atricapillus.*
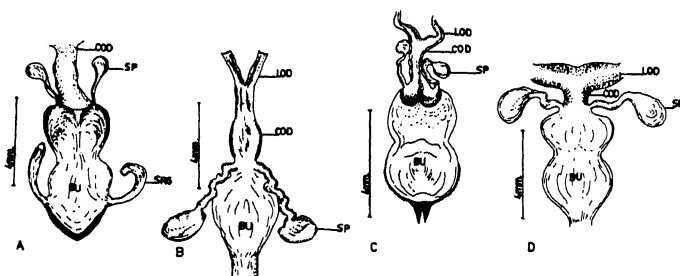
Figure 5. **A.** *E. plagiatus.* Absence of subrectal glands in **B.** *R. atkinsoni,* **C.** *I. armipes* and **D.** *P. armatissimus.*
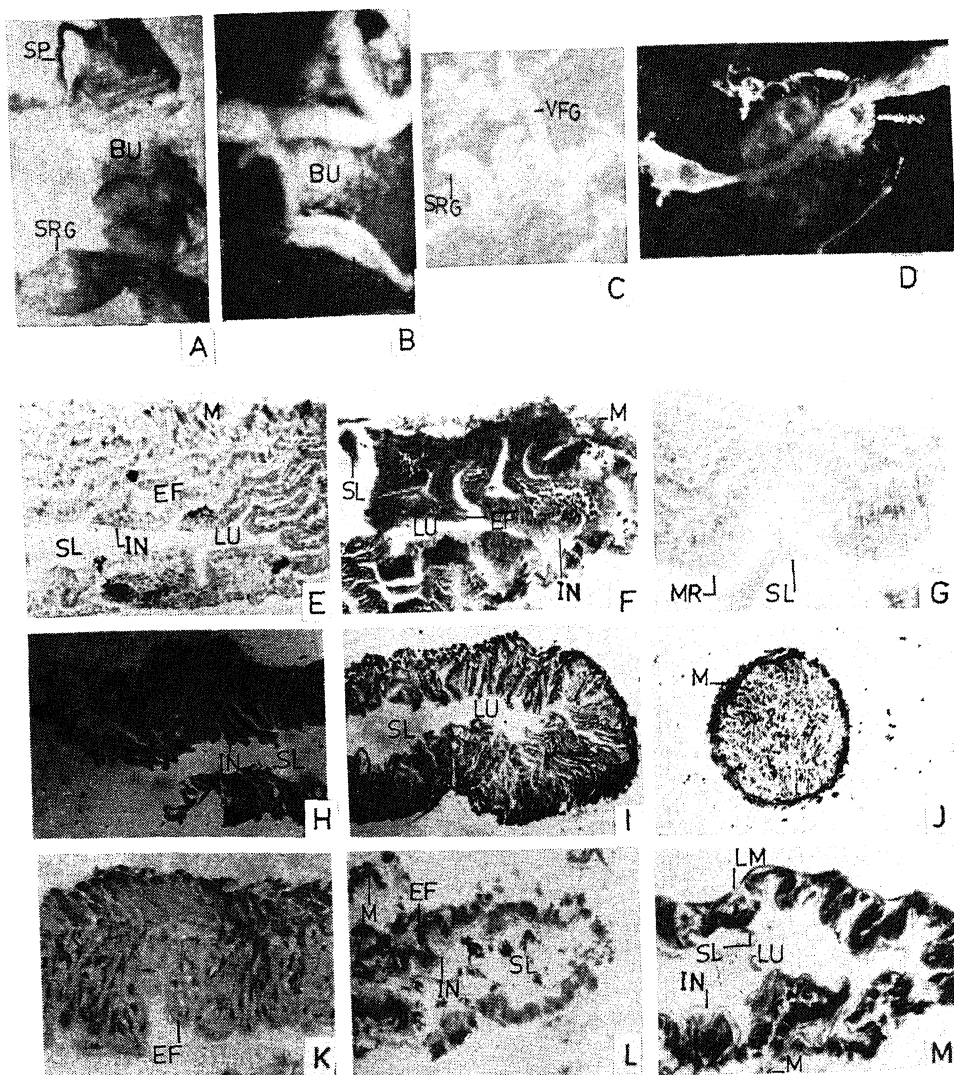
Figure 6. Female internal organs of reproduction. In situ. **A.** *R. fuscipes* (10 × 10x).
B. *R. kumarii* (10 × 10x). **C.** *L. guerini* (10 × 10x). **D.** *H. nigroviolaceus* (10 × 10x). **E.** LS
of the subrectal gland of *S. versicolor* showing glandular epithelium (20 × 10x). **F.** LS of
the subrectal gland of *R. marginatus* showing epithelial foldings projecting into the lumen
(20 × 10x). **G.** Enlarged view of the same gland showing the secretion of spumaline in the
merocrine fashion (40 × 10x). **H.** LS through the posterior region of the gland of
R. kumarii showing the strong circular muscle at the orifice (20 × 10x). **I.** LS of the same
gland showing epithelial folds and spumaline in the lumen (20 × 10x). **J.** TS through the
apical bulb of the gland in *R. kumarii* showing the non secretory phase (20 × 10x). **K.** LS of
the subrectal gland of *R. kumarii* showing the villi like processes (40 × 10x). **L and M.** LS
of the subrectal glands of *N. therasii* and *L. guerini* showing the spacious lumen and
spumaline (20 × 10x).

(*Abbreviations*: BU, Bursa; BUG, bursal gland; CM, circular muscles; COD, common
oviduct; EF, epithelial fold; GP, gonopore; IN, intima; LOD, lateral oviduct; LU, lumen; M,
muscle; MR, merocrine; SL, spumaline; SP, spermatheca; SRG, subrectal gland; VFG,
vermiform gland).

from the median bursal gland, a pair of voluminous sacs have also been traced. These structures are opening separately into the bursa.

3.2 *Histomorphology*

The histomorphology of the subrectal glands of *S. versicolor, R. marginatus, R. kumarii, N. therasii* and *L. guerini* has been examined. Histological sections show that they possess a secretory highly folded epithelium in the form of finger like processes projecting into the lumen both in *S. versicolor* (figure 6E) and in *R. kumarii* (figure 6K). The epithelial layer forms massive undulations in *R. marginatus* (figure 6F) and simple lobe-like inpushings in *N. therasii* and *L. guerini* (figure 6L, M). The compact but highly folded condition of the epithelial layer increases the secretory surface of the gland (figure 6J). The intra-epithelial space formed by the foldings of the epithelial wall is lined with chitinous intima and muscle bands so that during the peak secretory phase, the folds could remain fully extended and the lumen more spaceous (figure 6L, M). The wall of the gland throughout is provided with both circular and longitudinal muscles. The opening of the gland into the duct is very narrow and the wall at this site is heavily invested with strong circular muscle that act as a sphincter, regulating the release of the spumaline secretion (figure 6H).

There is evidence to suggest that the secretory activity of the gland is periodical, since the secretion of the gland at ovipositing and non-ovipositing periods indicate histologically different characters. The epithelium becomes secretory when the insect starts laying eggs. During the secretory phase, the nuclei become enlarged and the cells secrete in the merocrine fashion (figure 6G). Towards the end of the secretory phase, the epithelium remains obliterated while the lumen is stuffed with secretory material (figure 6L). The gland in *S. versicolor* contains a viscous clear secretion (figure 6E). The spumaline in *R. kumarii* is a homogenous clear secretion (figure 6H, I). A brownish secretion found in the subrectal gland of *R. marginatus* contains crystalline particles (figure 6G). In *N. therasii* and *L. guerini* the lumen is densely packed with coarsely granular material (figure 6L, M). The spumaline is found soluble in water and gradually gets hardened when smeared around the eggs.

4. Discussion

Glandular structures associated with the female reproductive system of Reduviidae, secreting spumaline, are commonly referred to as accessory glands. In Reduviidae paired ectodermal glands, restricted mostly to the harpactorine groups, have been variously named as colleterial glands (Kershaw 1909) and subrectal glands (Davis 1969).

Earlier reports on the occurrence of these glands were quite at variance. Kershaw (1909) described them as paired colleterial glands in *Sycanus croceovittatus*, having a common duct opening dorsally into the posterior region of the bursa. Scudder (1959) misinterpreted the same glands as paired spermathecal glands, Davis (1966) located the opening of such glands between the second valvulae and the styloids. Later, in (1969) he correctly traced the exit of a large bilobed sac in *Pselliopus cinctus* in the membrane between the styloids and anus and named it as subrectal gland. According to him these glands are having the function of scent production and considered them

as repugnatorial glands. Later, it was confirmed that the secretion of these two voluminous sacs to be of glutinous nature serving the purpose of adhering the eggs to the substratum.

Louis and Kumar (1973) have recorded a pair of yellowish accessory glands, opening independently of the common oviduct, in *Pisilus tipuliformis* and another elongated light pink coloured accessory gland in the harpactorine species *Margasus afzelli*. Earlier, Readio (1927) has reported that in *Acholla multispinosa* the secretion of such gland functioned both as cementing and protective substance. Later, Miller (1956) suggested that in *Isyndus heros* this material provides oothecal effect to the eggs. While describing the grooming behaviour of *Apiomerus crassipes*, Swadner and Yonke (1973b) reported that the spumaline was removed from the abdominal tip by the metatibial comb and deposited over the eggs.

However, the subrectal glands were reported to be absent in the genera *Acholla, Sinea, Fitchia* and *Rhaphidosoma* by Davis (1969) and in *Coranus subapterus* and *C. aegyptius*, by Cobben and Wygodzinsky (1975). Subsequently, in *Sinea coronata* Cobben and Wygodzinsky (1975) have traced a pair of subrectal gland. Davis (1969) has reported the occurrence of subrectal glands in several harpactorine tribes such as Phonolibinae, including a *Lophocephala* species; Tegeines and Diaspidines. Their absence was reported in Apiomerini and Ectinoderini. However in the latter, a similar structure that opens just below the styloids into the genital tract was not considered as subrectal glands by Davis (1969). In an ectrichochine species, *Glymmatophora*, a pair of voluminous sacs lying lateral to the genital chamber were labelled as Pseudospermathecae by Scudder (1959). These sacs may represent the Saccular structures described here in *H. nigroviolaceus* (Ectrichodiinae). Though these paired structures are labelled as subrectal glands because of their morphological resemblance with the subrectal gland its histomorphology was not studied in the present investigation. Haridass (1987) described that they are lateral expansions of the bursa, serving the purpose of storing eggs before oviposition.

In most of the earlier descriptions of the female accessory glands of Reduviidae mention has been made only of the accessory gland and for that reason the status of the subrectal gland remained unexplained for long. The designation of the same in several species has become more confusing when it was considered as Pseudospermatheca by Scudder (1959). Consequently, any interpretation based on the earlier literature on spermatheca and subrectal gland has become an arduous task. Therefore, in the present descriptions and illustrations the term subrectal glands has been found to be more appropriate to designate the spumaline secreting glands that predominantly occur in Harpactorinae.

Though the subrectal glands are found to have developed in varying degrees among the 15 species of Harpactorinae examined, they are significantly absent in the harpactorine species, *Irantha armipes*; *Rhaphidosoma atkinsoni* and in *Polydidus armatissimus* which also glue their eggs to the substratum. Only in the case of *Lophocephala guerini* which is considered here as an aberrant harpactorine species of western ghats, both the subrectal glands and bursal glands have been found in paired condition.

Anatomical and histological details of subrectal glands in *Arilus carinatus* were given by Barth (1961). The present observations on the histological preparations of subrectal glands reveal that the compact arrangement of glandular foldings that fill the central lumen is characteristic of harpactorine species confined to the tropical

rain forest, whereas in *L. guerini* and *N. therasii* of the tropical rain forest and scrub jungles the lumen remains quite spacious because of the presence of simple glandular epithelial lobes. Thus the histomorphology of the gland also varies in accordance with the requirement of spumaline for their specific oviposition strategy. Though the subrectal glands and bursal glands are functionally colleterial, the histomorphology, secretory phases and spumaline itself are convincing evidences for their hetero-genicity. Since the subrectal gland occurs commonly in Harpactorinae and the bursal glands in other reduviids, they may have evolved independently of each other.

Salkeld (1972) has suggested the hygroscopic property for the viscous material which covers the veil. It gets dried up and shrinks under vacuum and regains its original state when returned to atmosphere. Hinton (1961) suggested that in *Nepa cinera* L. the viscous material covering a part or the egg in whole may function as a humidity regulator. Cobben and Wygodzinsky (1975) observed that the gluing material in *Sinea spinipes* is less adhesive than that in *Sinea diadema*, similarly in the present study too spumaline secreted by *Endochus cingalensis* appears more sticky whereas in *E. inornatus* spumaline gets tanned into a hard coating around the eggs as in most of the other harpactine eggs. According to Miller (1956) and Swadner and Yonke (1973a, b) the eggs on the periphery of the egg mass and loosely deposited eggs are more susceptible to parasite attack while the eggs that are at the centre and those heavily coated with spumaline are less susceptible to parasites. In the present investigation too most of the peripheral eggs in a batch of eggs of *R. kumarii* collected from the field have been found parasitized by a dipteran parasite. Presence of several exit holes found on the egg shell of *Rhaphidosoma atkinsoni* usually smeared with little spumaline on the egg surface suggests the probability of these eggs being hyper parasitized as recorded in *Arilus cristatus* by Swadner and Yonke (1973a).

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Preliminary studies on the venom of three Indian spiders

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Abstract. Pure venom from 3 Indian spiders was extracted by electrical stimulation. The average amount of venom yielded per spider and the average protein content in the venom were evaluated. The venom proteins and free amino acids have been separated by electrophoretic and chromatographic techniques respectively. Nine major fractions were separated from the wolf spider, *Lycosa indagastrix* and 7 from funnel web spider *Pleisiophrictus collinus* and the common house spider *Heteropoda venatoria*. Each venom had a distinct electrophoretic pattern. Chromatographic studies revealed that proline was the only free amino acid common to all the 3 venoms.

Keywords. Venom; venom proteins; free amino acids.

1. Introduction

Spider venom has received considerable attention by a number of workers because of its potency and their clinical manifestations in man. However, the major work done was on a few medically important genera like the black widow spider, *Latrodectus* by Maretic (1966), Mccrone (1964), Mccrone and Hatala (1968), the brown recluse spider, *Loxosceles* by Smith and Mick (1969), Mowis and Russel (1975) and Morgan (1969), the Sydney funnelweb spider *Atrax* by Wiener (1957, 1961) and Gilbo and Coles (1964) and the wolf spider *Lycosa* and the banana spider, *Pheneutria* by Fischer and Bohn (1957), as they possess potent venoms which can produce severe envenomation in man.

In view of the fact that *Pleisiophrictus collinus*, *Lycosa indagastrix* Walck 1837 and *Heteropoda venatoria* Linn 1766 are widely distributed in and around the Madras city and since cases of their bites are quite common. Although it has been well documented that the envenomation by spiders could cause severe necrosis in humans, research on the venom of spiders is limited mainly due to the difficulty in capturing the large number of spiders to produce adequate quantity of venom, needed for the research, and in obtaining pure venom from these spiders, owing to their small gland size. This communication presents some quantitative data on the collection of venom and a comparative electrophoretic and chromatographic pattern of venom proteins and free amino acids.

2. Materials and methods

The specimens used in the present investigation were collected periodically within the 363-acre scrub jungle of the Madras Christian College and also from the agricultural fields and deserted houses in the neighbouring villages on the outskirts of

the Madras city. *P. collinus* the common funnel-web spider a mygalomorph, and *H. venatoria,* the common house spider and *L. indagastrix* the wolf spider are araneomorphs, chosen for the present study.

2.1 *Extraction of spider venom*

Among the methods used for obtaining spider-venom the most common ones are the surgical removal of the venom glands, or the use of an electric stimulator (Smith and Mick 1969). The former however presents two problems i.e. each spider can be used only once, and moreover the material obtained is a mixture of venom along with the gland tissues. Hence in the present investigation venom was extracted from unanaesthetised spiders by using an electrical stimulator, improvised in the laboratory, but based essentially on the apparatus designed by Morgan (1970). The stimulator was successful in that the electric shocks delivered were sufficient to stimulate spiders for the extraction of venom quickly.

The stimulator used consists of batteries, wired to an automobile ignition coil and a flat metal plate for mounting the spider. A high voltage direct current is discharged through the wire of the ignition coil to the copper stimulator. The required voltage can be set with the rotary switch (figure 1a).
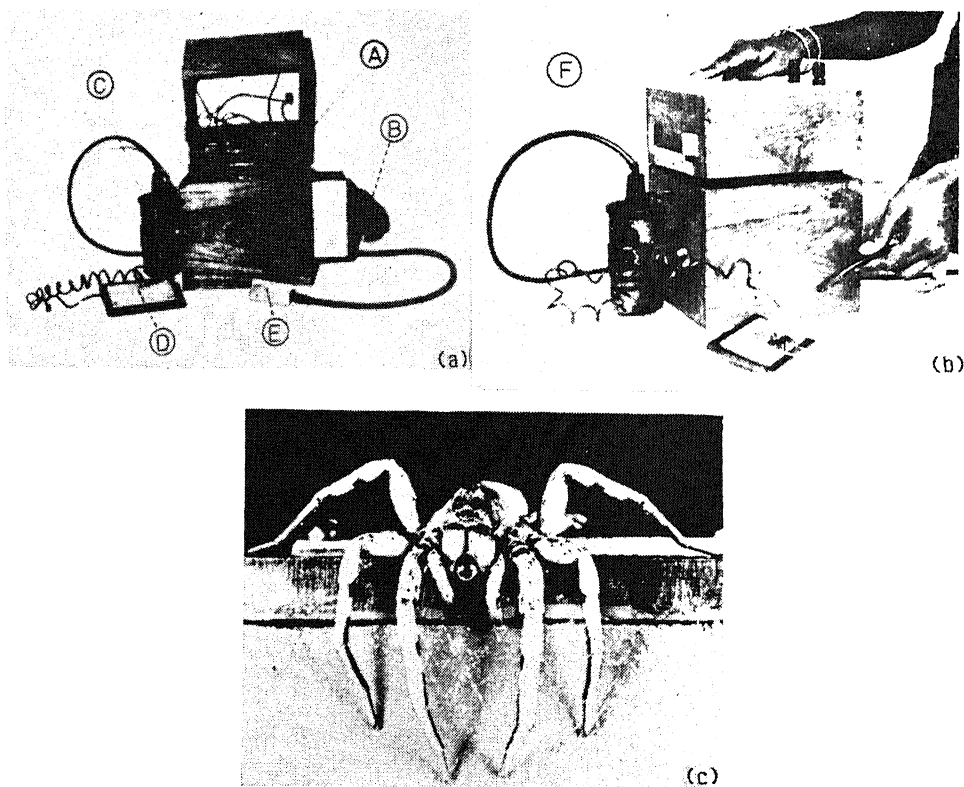
Figure 1. **a.** Electric stimulator for extraction of venom. (A, Batteries; B, rotary switch; C, automobile ignition coil; D, metal plate; E, stimulator). **b.** Process of extracting venom from a spider. **c.** A droplet of venom obtained in stimulating a spider.

For venom extraction, an unanaesthetised spider was fixed to the metal plate, with its cephalothorax projecting slightly over the edge of the plate. The stimulator was then placed on the cephalothorax, just posterior to the eyes (figure 1b). Closing and opening the switch produced an electrical impulse in the form of a spark. A few such electrical stimuli were sufficient to produce maximum contraction of the venom glands, whereby a small droplet of venom was exuded out of the fangs (figure 1c).

2.2 *Determination of protein*

Protein concentration in the venom was assayed according to Lowry *et al* (1951). All absorbance readings were taken in a Kontron-Uvikon spectrophotometer.

2.2a *Polyacrylamide gel electrophoresis*: For the purpose of studying the nature of the proteins of spider venom, electrophoretic method of Davis (1964) was employed. Electrophoresis of the venom was carried out at 4°C in a 7·5% polyacrylamide gel, set in a disc gel electrophoresis (Broviga) with the conventional Tris glycine buffer at pH 8·3. 0·1 ml of venom reconstituted with distilled H_2O was loaded into each slot. Maximum resolution was achieved using 4 mA gel tube at 100 volts for 60–70 min. The gel were later stained with 0·2% aqueous solution of Coomassie brilliant blue and destained with 7% acetic acid.

2.2b *Densitometric analysis*: Densitometric scanning of proteins was carried out by scanning the gels with the help of the Chromoscan Schimadzu cs-910, Dual wave length thin-layer chromatography scanner.

2.3 *Chromatographic separation of free amino acids*

Free amino acids were separated from spider venom using a uni-directional paper chromatography. The venom sample was mixed with 2 ml of 80% ethanol for precipitation of the proteins and centrifuged at 11,000 g for 5 min. Three volumes of chloroform was then added to the supernatant to remove the precursors of lipids. The aqueous layer formed at the top was used for spotting. Butenol, acetic acid and water was used as the solvent, in the ratio 12:3:5. The amino acids were identified using known R_f values of the standard run under similar conditions.

3. Result and discussion

Pure venom for the present study was obtained on electrical stimulation (4–6 volts) by a variable (0–12) volt transforer, which permitted slow extrusion of venom from the fangs into a microcapillary tube. The venom was a clear, highly viscous droplet of fluid, with a tendency to dry fast.

3.1 *Venom quantity*

The average amounts of venom obtained per spider of each species are listed in table 1. *P. collinus* being less aggressive and less irritable, yielded very low quantity of venom ($0·29 \pm 0·02\ \mu l$/spider) as against *L. indagastrix* which yielded a significantly larger

Table 1. Average yield and protein analysis of venom* in the spiders.

Species	Average yield of venom (μl/spider)	Protein concentration (μg/μl)
L. indagastrix	0·65 ± 0·03	205·38 ± 7·97
H. venatoria	0·38 ± 0·02	251·15 ± 8·25
P. collinus	0·29 ± 0·02	274·22 ± 8·56

Mean ± SD.
*Each sample is obtained by pooling venom from 10 mature spiders.

quantity of venom (0·65 ± 0·03 μl/spider) and *H. venatoria* that yielded 0·38 ± 0·02 μl/ spider. Earlier reports on venom collection were questionable due to contamination by stomach contents and other secretions. The use of a stimulator, in the present study eliminates these contaminations and facilitates reproducibility. The methods of extraction were effective among all the spiders tested, less than 5% failed to give measurable quantity of venom. On comparing the average yield of venom, with the data referring to the much dreaded *Loxoceles reclusa* by Morgan (1969) and Smith and Micks (1969), it appears that the common house spider *H. venatoria* has a similar quantity of venom, but the quantity of venom in *L. indagastrix* is comparatively much higher. The venom may vary quantitatively and qualitatively from species to species and may also vary even within an individual, at different times of the year, or under different environmental conditions or even with the age (Morgan 1969; Keegan *et al* 1960). In the present study, although variations in venom volumes were observed during different stages of development, there was no significant difference in the quantity of venom yielded by either of the sexes.

3.2 Venom proteins

The protein concentration of the venom (μg/μl) is listed in table 1. Though the average protein content in the venom of *L. indagastrix* is higher (133·00 ± 2·68 μg/ spider) and that of *P. collinus* is as low as 80·33 ± 2·34 μg/spider, the concentration of proteins per ml of the venom is the highest in the mygalomorph spider (274·22 ± 8·56 μg/ μl). The concentration of the toxic principles or the protein in the venom of the house spider is found to be 251·15 ± 8·25 μg/μl and that of the wolf spider 205·38 ± 7·97 μg/ μl, against the copious amount of venom secreted by it.

Based on the finding of Hodgon and Frank (1980), who suggested that proteins play an important role in venom toxicity and that these roles assume different importance in various species of venomous animals, a qualitative analysis of spider venom proteins were attempted using disc-gel electrophoresis. Since venom is known to be unstable with respect to its protein component (Schottler 1951), pure venom used for the assay was fresh. A typical stained polyacrylamide gel electrophoretic pattern of the venom along with their respective scan patterns are presented in figure 2. The total number of fractions, their protein content and the relative proportion (%) are given in table 2. Though all gel columns were run at the same time and under the same conditions, variations were observed in the venom proteins which could be attributed to the wide range of toxicities observed in spiders. Such toxic principles are capable of producing one or more deleterious changes in several
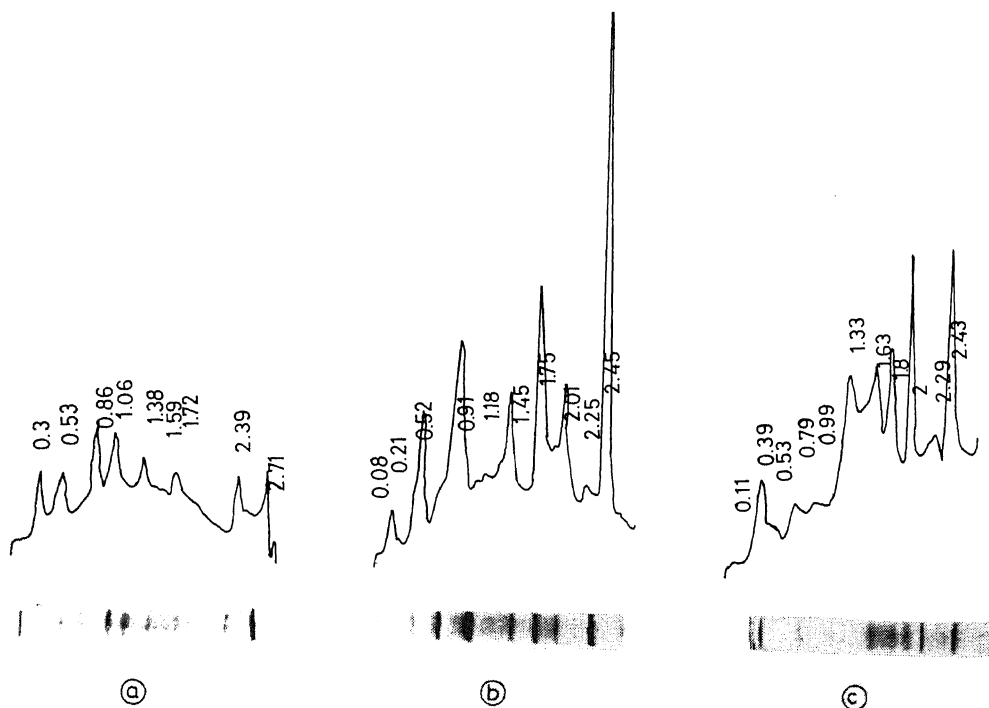
Figure 2. A typical stained polyacrylamide gel electrophoretic pattern of the venom along with their respective scan patterns. **a.** *Pleisiophrictus collinus.* **b.** *Heteropoda venatoria.* **c.** *Lycosa indagastrix.*

organ systems or tissues and are even capable of provoking some of these changes concurrently.

For the purpose of convenience and to avoid ambiguity, the electrophoretic patterns seen in the running gels are broadly divided into 3 categories (table 2) of the 9 major fractions observed in the scan pattern of the venom of *L. indagastrix*, 3 slow moving cathodic proteins were seen near the point of application of the venom. Similarly of the 7 major fractions in the venom of the house spider 3 of them were slow fractions. In the mygalomorph, *P. collinus* venom, only two cathodic fractions were noticed.

However, a few variations were observed in gel patterns of any particular species. This intraspecific variations could depend on the stage in the regeneration cycle at which the venom sample is obtained. The labile nature of venom is especially true with regard to liquid venom (Marsch 1974). As venom secretion cannot cope with the demands at the moment of need, the spider has to store venom in the lumen and possibly in the secretory cells of the gland. Hence venom obtained on the first day of milking had possibly been subject to autolysis or other breakdown process. With subsequent milking, fresh venom is produced and the electrophoretic pattern stabilises.

Although, the venoms appear to have the same basic nature, electrophoretic studies indicate that there are qualitative differences in them. However several common protein fractions were observed in the venom samples of the 3 species. Two

Table 2. Qualitative analysis of proteins in the venom of spiders.

| | | | Electrophoretic fractions | | |
| | | | Proximal (−) | | |
Species	Volume of venom (μl)	Total protein content (μg)	No. of fractions	Relative proportion (%)	Protein content (μg)
L. indagastrix	4·5	920·77	3	30·364	279·58
H. venatoria	4·5	1068·75	3	35·581	380·27
P. collinus	4·5	1285·71	2	16·102	207·03

| | Electrophoretic fractions | | | | |
| | Middle | | | Distal (+) | |
Species	No. of fractions	Relative proportion (%)	Protein content (μg)	No. of fractions	Relative proportion (%)	Protein content (μg)
L. indagastrix	3	53·694	494·40	4	15·770	145·21
H. venatoria	3	35·936	384·07	4	28·491	304·50
P. collinus	3	40·659	522·6	4	43·239	555·93
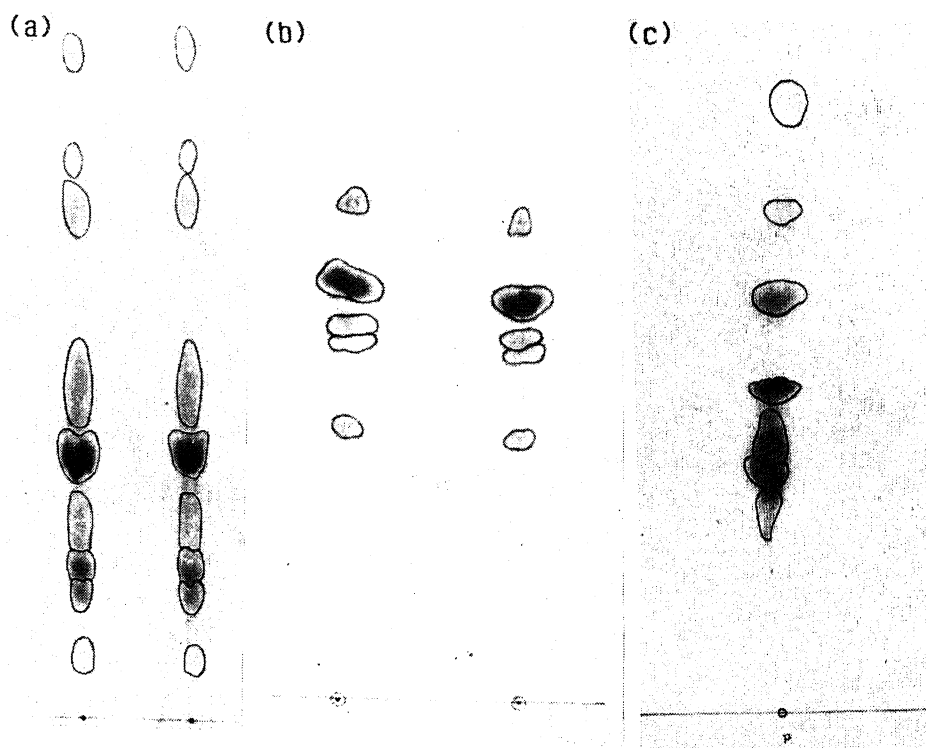
Figure 3. Free amino acids in the venom. **a.** L. indagastrix. **b.** H. venatoria. **c.** P. collinus.

similar cathodic fractions and two similar anodic fractions of relatively similar mobilities were observed in the venom of *L. indagastrix* and *H. venatoria*. Likewise, one similar distal fraction was noticed in the venom of the mygalomorph spider *P. collinus* and *L. indagastrix* and another similar anodic fraction in the venom of *H. venatoria* and *P. collinus*. The presence of such common antigens in the venom of dreaded species may enable one to prepare antivenin against one that could neutralise the lethal effects of the venom of other species.

3.3   *Free amino acids of the venoms*

A comparison of the free amino acids in the venoms of the spiders were presented in figure 3. *Lycosa* venom was found to contain 9 different amino acids — cysteine, lysine, histamine, glutamine, proline, Iso-leucine, methionine, tryptophan and glutamic acid. *P. collinus* venom contained 6 free amino acids and *H. venatoria* venom had 4 free amino acids, proline being the only amino acid common to all the venoms.

Hence it is evident from the foregoing analysis that the venoms of the 3 common Indian spiders present unique and a distinct pattern of proteins and free amino acids which could probably account for the differences in their clinical manifestation following envenomation by the spiders.

Acknowledgement

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Population biology and reproductive strategy of *Dichogaster bolaui* (Oligochaeta: Octochaetidae) in two tropical agroecosystems

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Abstract. Comparative biology of the worm *Dichogaster bolaui* (Michaelsen) in a pasture and compost pit has been reported. Important findings are: (i) restriction of more than 84% of *Dichogaster bolaui* population to upper 10 cm of the soil depth indicating surface living habit; (ii) attainment of a peak population of 12617 worms/m²; (iii) a zero population during summer revealing environmental drasticity (soil temperature >31°C, soil moisture <1 g%) and yearly recolonisation of worms; (iv) habitat variation in the reproductive strategy with an unimodality in pasture and bimodality in compost pit; and (v) pre-emergence of juveniles followed by post cocoon peak indicating population survival through cocoons during summer.

Keywords. *Dichogaster bolaui*; unimodality; bimodality; reproductive strategy; life cycle; vertical stratification.

1. Introduction

There is a great paucity of information on the biology of tropical earthworms (Dash 1978; Lavelle 1978). There are about 500 species of earthworms in India (Julka 1976). Utilisation of suitable biological agents in vermitechnological programme is largely dependent on the width of our knowledge on the autecological studies of these organisms. A comprehensive programme for understanding the environmental regulation of life cycle strategy in tropical earthworms has been taken up in different agroecosystems. Dash and Patra (1977), Senapati *et al* (1979), Dash and Senapati (1980) and Sahu and Senapati (1986) have reported the activity of earthworms in lowland, upland and plain pasture ecosystems of Orissa. Impact of large herbivore grazing and thermohydric stress on tropical earthworms has been studied by Senapati and Dash (1981, 1983, 1984). Out of the 31 earthworm species reported by Julka and Senapati (1987), *Dichogaster bolaui* (Michaelsen) is the smallest with a maximum of about 40 mm in length, 1 mm in diameter and a dry weight of 7 mg with 80% of tissue moisture content. Interspecific interaction studies carried out on tropical earthworms indicate that *D. bolaui* has great potentiality in the biodegradation of organic residues (Senapati B K, Sahu S K and Pani S C, unpublished results). However, no attempt has been made to understand the orientation of life cycle strategy of detrivorous earthworms, like *D. bolaui* in different organic waste disposal sites. The present paper deals with the population biology and reproductive strategy of *D. bolaui* in two tropical agroecosystems receiving organic wastes.

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2. Study site and climate

Out of the two sites, one is an upland grazed pasture receiving kitchen wastes from Sambalpur University canteen from July–October at Jyotivihar and the other one is a dung deposit site receiving dung from a nearby farm having 50 cows and buffalows at Ainthapali (Sambalpur). Geographically the study sites are situated at 21° 25′ N latitude, 83° 52′ E longitude and about 160 m above the mean sea level. Detailed study has been made from a plot of 10 × 10 m area at both the sites, each divided into 400 subplots of 0·5 × 0·5 m² area. The study sites lie in the arid-humid agroclimatic belt (Lenka 1972). The climate is broadly divided into 3 seasons: winter (October–February), summer (March–mid June) and rainy (mid June–September). Climatological parameters have been measured by standard methods (Senapati *et al* 1979; Dash and Senapati 1980; Senapati and Dash 1981, 1983). Comparative characteristics of both the study sites relating to physical, chemical and biological parameters have been given in table 1. Hereafter the pasture site receiving kitchen waste and dung deposit site will be designated as pasture and compost pits, respectively.

3. Materials and methods

The present study was carried out at fortnightly intervals for about a year. At pastures site, sampling took place from July 1984 to June 1985 and from November 1985 to October 1986 at the compost pit site. The dates·were fixed between 13th and 15th for the second week sampling and between 28th and 30th for the fourth week sampling every month. During each sampling, 5 random samples were taken from an area of 25 × 25 × 20 cm separately from each study site (Snedecor and Cocharane 1967; Dash and Senapati 1980). Earlier works of Dash and Patra (1977), Senapati *et al* (1979), Dash and Senapati (1980) and Senapati and Dash (1981, 1983) on tropical earthworms from various agroecosystems had standardised methods for sampling, collection, preservation and analysis of cocoons and worms. *D. bolaui* was dominant in both number and biomass (>80%) in both the study sites. On the basis of length and clitellar development, *D. bolaui* worms were divided into 3 age classes. They were (i) juvenile (<1 cm, non-clitellate), (ii) immature (>1 cm but <2 cm, non-clitellate) and (iii) adult (>2 cm, clitellate). In the pasture, cocoons of *D. bolaui* were available during the study period and rate of reproduction was calculated from the ratio of total cocoons to adults and total juveniles to adults. Sahu and others (Sahu and Senapati 1987; Senapati B K, Sahu S K and Pani S C, unpublished results) have indicated the possibility of utilising juvenile: adult ratio in the absence of cocoon data. In the compost pit, inspite of all precautions and care, cocoons were not detected. In this changed situation the rate of reproduction was calculated from the ratio of total juveniles to adults.

4. Results

4.1 *Population size and structure*

Table 2 gives the population density and biomass of earthworms in various world agroecosystems. Table 3 shows the age structure of *D. bolaui* in two agroecosystems.

Table 1. Comparative characteristics of physical, chemical and biological parameters in two agroecosystems.

Parameter	Pasture site (1984–1985)	Compost pit site (1985–1986)
Solar radiation (average, 10^3kJ/m^2/month)	507·19	507·19
Rain fall (total, mm)		
Winter	114·8	145·4
Summer	104·3	114·3
Rainy	1545·2	1277·9
Air temperature (°C)		
Winter	11·67–33·95	10·80–31·74
Summer	18·73–42·71	17·91–42·14
Rainy	23·76–33·91	23·51–32·85
Relative humidity (%)		
Winter	26·56–97·60	28·67–98·25
Summer	14·87–94·67	17·60–94·60
Rainy	42·40–98·19	51·74–97·93
Soil/dung moisture (g%) (at 0–10 cm depth)		
Winter	4·8–16·0	9·1–50·1
Summer	0·9–17·2	0·3–16·0
Rainy	15·5–25·3	49·7–64·6
Soil/dung temperature (°C) (at 0–10 cm depth)		
Winter	18·2–27·5	17·8–30·2
Summer	24·2–30·1	22·0–32·1
Rainy	27·1–28·8	27·0–31·1
Organic matter (g%)		
0–5 cm	10·0	80·0
6–10 cm	6·5	75·0
11–15 cm	4·0	65·0
16–20 cm	2·0	50·0
21–40 cm	1·0	40·0
Earthworm species	*Dichogaster bolaui** *Lampito mauritii*	*Dichogaster bolaui** *Perionyx excavatus*
Vegetation	*Cynodon dactylon** *Spermacocea hispida*	—

*Dominant species.

Figure 1 depicts the fortnightly density of total *D. bolaui* population in two agroecosystems.

An average population of about 1447/m^2 and 1665/m^2 was noted in the pasture and compost pit, respectively. A zero population during summer months at both the sites indicated rebuilding of worm population each year. Peak population (nos/m^2) of 8038 in the pasture and 12617 in the compost pit noted in this study is the highest density reported so far. However, the highest value for worm biomass (g live wt/m^2)

Table 2. Population density (nos/m²) and biomass (g live wt/m²) of earthworms in various world agroecosystems.

Habitat	Population density	Population biomass	Locality	Extraction method	Reference
Savana					
Low laying wet savana	180–340	39–57	Lamto (Ivory coast), Africa	H	Lavelle (1977)
Grass savana	202		Lamto (Ivory coast), Africa	H	Lavelle (1978)
Shrub savana	350		Lamto (Ivory coast), Africa	H	Lavelle (1978)
Shrub savana	400		Lamto (Ivory coast), Africa	H	Lavelle (1978)
Grasslands					
Natural grasslands					
Pasture (base-rich grassland)	389–470	52–110	Westmoorland, UK	H	Svendsen (1957)
Pasture (base-rich grassland)	390	56	Bardsey Island	H	Reynoldson et al (1955)
Pasture (base-rich grassland)	481–524	112–120	N Wales	H	Reynoldson (1955)
Pasture (upland protected)	122–387	20–78	Sambalpur, Orissa, India	H and WS	Senapati and Dash (1981)
Pasture (lowland protected)	64–800	6–60	Berhampur, Orissa, India	H	Dash and Patra (1977)
Human interfered grasslands					
Pasture (upland grazed)	75–272	12–70	Sambalpur, Orissa, India	H and WS	Senapati and Dash (1981)
Pasture (upland grazed)	0–140	0–26	Jyotivihar, Orissa, India	H	Pradhan and Mishra (1986)
Arable land Paddy field (upland)	32–1399	2–62	Sambalpur, Orissa, India	H and WS	Pani and Senapati (unpublished results)
Paddy field (lowland)	0–246	0–25	Sambalpur, Orissa, India	H and WS	Pani and Senapati (unpublished results)
Organic waste deposit sites					
Pasture receiving kitchen waste	0–8038	0–66·2	Jyotivihar, Orissa, India	H and WS	Sahu and Senapati (1986)
Farmyard manure garden	15–625	–	Bangalore, Karnataka, India	H	Kale and Krishnamoorthy (1982)
Dung deposit site	0–12617	0–51·4	Sambalpur, Orissa, India	H and WS	Present work
Kitchen waste deposit site	600	–	Rajgangpur, Orissa, India	H	Julka and Senapati (1987)
Wash basin waste receiving site	50–500	–	Jyotivihar, Orissa, India	H	Julka and Senapati (1987)
Straw thatched roof drain site	800	–	Ladukhai, Orissa, India	H	Julka and Senapati (1987)

H, Hand sorting; WS, wet sieving.

Table 3. Age structure of *D. bolaui* in two agroecosystems.

| Month (week) | Pasture site (1984–1985) | | | | Compost pit site (1985–1986) | | | |
	Juvenile worms (J)	Immature worms (I)	Adult worms (A)	Total worms (TW)	Juvenile worms (J)	Immature worms (I)	Adult worms (A)	Total worms (TW)
Jul. (IV)	1193	1466	372	3031				
Aug. (II)	1582	5585	871	8038				
(IV)	1208	2571	644	4423				
Sep. (II)	78	3242	1132	4452				
(IV)	238	120	676	1034				
Oct. (II)	118	473	474	1065				
(IV)	0	40	80	120				
Nov. (II)	—*	—	—	—				
(IV)	0	0	39	39	361	1579	199	2139
Dec. (II)	—	—	—	—	1207	4777	554	6538
(IV)	0	0	39	39	268	1894	211	2373
Jan. (II)	—	—	—	—	17	233	24	274
(IV)	0	0	39	39	41	95	36	172
Feb. (II)	—	—	—	—	0	0	17	17
(IV)	0	0	39	39	0	0	0	0
Mar. (II)	—	—	—	—	—	—	—	—
(IV)	0	0	0	0	0	0	0	0
Apr. (II)	—	—	—.	—	—	—	—	—
(IV)	0	0	0	0	0	0	0	0
May (II)	—	—	—	—	—	—	—	—
(IV)	0	0	0	0	0	0	0	0
Jun. (II)	0	40	80	120	—	—	—	—
(IV)	118	473	119	710	23	98	63	184
Jul. (II)					342	308	116	766
(IV)					315	1570	233	2118
Aug. (II)					2514	8823	1280	12617
(IV)					387	1548	212	2147
Sep. (II)					240	491	228	959
(IV)					59	123	111	293
Oct. (II)					29	93	134	256
(IV)					239	206	156	601

*Sampling not done.

was reported from the base rich grasslands (table 2). Population structure constituted 11–39% of juveniles, 12–73% of immatures and 11–100% of adults during 1984–1985 study period at the pasture. In the compost pit the population structure comprised 6–45% of juveniles, 34–85% of immatures and 9–100% of adults during 1985–1986 study period. This shows that the youngest age group i.e. juveniles forming the smallest component of the total population, indicate rapid transformation and/or high mortality and/or discontinuous reproduction resulting in an unstability in the age structure. In the compost pit total worm population showed one peak during December II week 1985 with 6538 worms/m² and another peak during August II week 1986 with 12617 worms/m² (table 3). The higher peak is associated with rainy season and lower peak with the postrainy season. This peak population value is about 57% higher than the highest density (8038/m²) of *D. bolaui* noted in the pasture site. Higher nutrient quality and longer activity period might be the cause for increased population density in the dung pit.

Figure 1. Fortnightly density of total *D. bolaui* population in two agroecosystems.

Table 4. Correlation coefficient values of different environmental parameters with total *D. bolaui* density.

	Total *D. bolaui* worm density	
Parameter	Pasture site	Compost pit site
Solar radiation	−0·458[a]	−0·526[b]
Rainfall (total)	0·177[c]	0·158[c]
Relative humidity (average)	0·598[d]	0·325[a]
Air temperature (average)	0·091[c]	−0·077[c]
Soil temperature (0–10 cm, average)	0·324[c]	0·014[c]
Soil moisture (0–10 cm, average)	0·719[e]	0·468[b]

[a, b, d, e] Significant at 0·2, 0·05, 0·02 and 0·01 respectively. [c] Not significant at 0·2.

4.2 Population distribution and environmental regulation

Table 4 gives the correlation coefficient values of different environmental parameters with total *D. bolaui* density and figure 2 shows the vertical distribution of *D. bolaui* population in two agroecosystems studied.

Environmental parameters fluctuated more drastically at the pasture compared to the compost pit. Soil moisture and solar radiation showed a positive and negative correlation with the total monthly worm density, respectively for both the sites (table 4). About 84 and 16% of the total worms were stratified at 0–10 and 11–15 cm of soil depth at the compost pit site whereas 96 and 4% of the worms remained at 0–10 and 11–15 cm of soil depth at the pasture site. Juvenile and immature worms were mostly restricted to 0–5 cm of soil depth. Thus the whole population was mostly confined to upper 10 cm of the soil profile indicating highest degree of exposure to the environ-

Figure 2. Vertical distribution of *D. bolaui* population in two agroecosystems.

mental fluctuations. Dynamics of total worms (nos/m²/fortnight) showed unimodal and bimodal form of population peak at pasture and compost pit site, respectively (table 3, figure 1). Diapause coils of worms were obtained during November–February in the pasture site and during February in the compost pit site. However these diapause coils were not available after February indicating death and destruction of the diapaused worms resulting in zero population during summer months. Thus there is a possibility of yearly recolonisation of the worms to the study sites from the surrounding wet habitats and/or due to hatching of cocoons survived through summer stress.

4.3 *Reproductive strategy*

Table 5 gives the rate of reproduction and figure 3 shows the life cycle of *D. bolaui* in two agroecosystems.

The cocoon of *D. bolaui* is lemon yellow, slightly oval in shape and measures about 2 mm in length and 1·25 mm in width with a distinct ornamentation at the hatching side. Usually 1–2 juveniles hatch out from the cocoon and the incubation period is about one week. Although cocoons were obtained from the pasture site, they were not available from the compost pit site. Deep coloration of the dung along with high organic matter (>70%) provided a very inappropriate background for cocoon identification. In the pasture site the cocoons were available from June IV week till October II week (table 5). The peak density of 4956 cocoons/m² was noted during II week of September 1984 when density of adult worms were 1132/m². Thus the rate of reproduction (total cocoon: total adult) came to be around 4·38. The ratios of total cocoons to adults and total juveniles to adults in all the sampling occasions did not show significant difference at 0·05 level of significance ($t = 0.22$, $n = 16$) indicating the use of juvenile: adult ratio as an alternate method of calculating rate of reproduction in the absence of cocoon. Emergence pattern of the worm was unique because the

Table 5. Rate of reproduction in *D. bolaui* at two agroecosystems.

| Month (week) | Pasture site (1984–1985) | | | Compost pit site (1985–1986) |
| | Total cocoons (TC) | Rate of reproduction | | Rate of reproduction |
		TC/A	J/A	J/A
Jul. (IV)	0	0·00	3·21	
Aug. (II)	76	0·09	1·82	
(IV)	645	1·00	1·88	
Sep. (II)	4956	4·38	0·07	
(IV)	915	1·35	0·35	
Oct. (II)	78	0·17	0·25	
(IV)	0	0·00	0·00	
Nov. (II)	—	—	—	
(IV)	0	0·00	0·00	1·81
Dec. (II)	—	—	—	2·18
(IV)	0	0·00	0·00	1·27
Jan. (II)	—	—	—	0·71
(IV)	0	0·00	0·00	1·14
Feb. (II)	—	—	—	0·00
(IV)	0	0·00	0·00	0·00
Mar. (II)	—	—	—	—
(IV)	0	0·00	0·00	0·00
Apr. (II)	—	—	—	—
(IV)	0	0·00	0·00	0·00
May (II)	—	—	—	—
(IV)	0	0·00	0·00	0·00
Jun. (II)	0	0·00	0·00	—
(IV)	39	0·33	0·99	0·37
Jul. (II)				2·95
(IV)				1·35
Aug. (II)				1·96
(IV)				1·83
Sep. (II)				1·05
(IV)				0·53
Oct. (II)				0·22
(IV)				1·53

J, Juvenile; A, adult.
'*t*' test between TC/A and J/A in pasture site not significant at 0·05 level.

cocoon peak (September II week, 1984) was observed after the juvenile peak (August II week, 1984). Pre emergence of juveniles followed by post cocoon peak might be an indication of population survival through cocoons during summer. The rate of reproduction (total juvenile: total adult) of *D. bolaui* at the compost pit site, reached 2·18 during December II week and 2·95 during July II week indicating two reproductive peaks. Thus two peaks in the compost pit and single peak in the pasture site indicated bimodality and unimodality of *D. bolaui* earthworm, respectively. The time lag between the emergence of juvenile from the cocoon and their transformation into adult till production of cocoons, is known as the duration of life cycle. Considering the peak rate of reproduction, peak juveniles and peak population the probable duration for completion of *D. bolaui* life cycle might be about 4–5 months in the pasture site and 4–8 months in the compost pit site (figure 3). The expected duration that an individual is supposed to live is known as life span.

Pasture site receiving kitchen waste   Dung deposit site

■ A , ▣ B , ● C , ○ D , □ E , ▤ F , ▥ G , ▲ H , ★ I , ★★ J
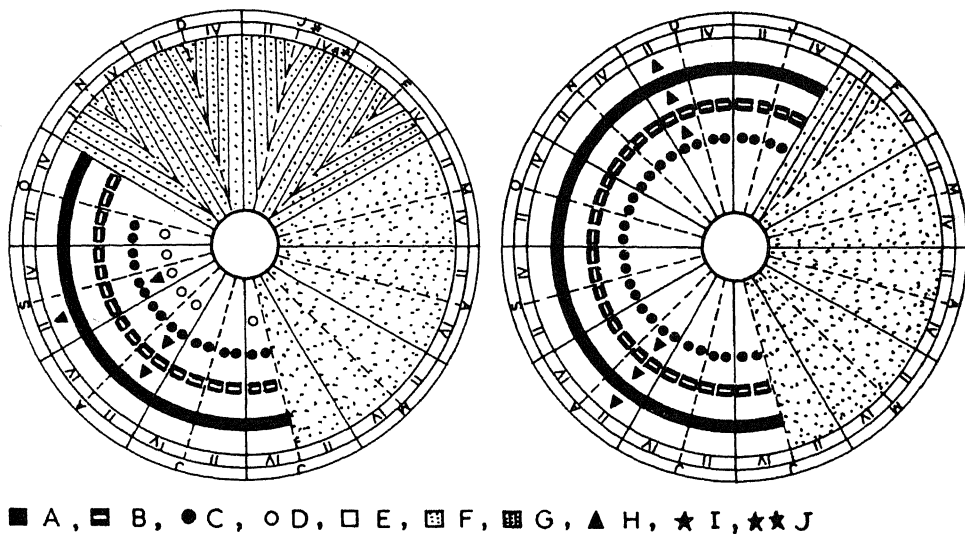
Figure 3. Life cycle of *D. bolaui* in two agroecosystems.
(A, Adult; B, immature; C, juvenile; D, cocoon; E, active period; F, worms not available; G, inactive period; H, peak number; I, month; J, week).

Since *D. bolaui* population reached zero level and showed annual recolonisation of worms, so the duration of the life cycle might be equal to the life span. This needs verification in laboratory culture.

5. Discussion

5.1 *Population distribution, size, structure and environmental regulation*

D. bolaui is a cosmopolitan species (Gates 1972; Julka and Senapati 1987). In India the worms show peregrinity (wide occurrence) (Stephenson 1923; Bahl 1950). However, excepting taxonomic details and places of collection, very little information is available on the ecology of this widely distributed worm. Sahu and Senapati (1986) first published the details of population biology and secondary production of the worm from a pasture ecosystem. Population density of $12617/m^2$ for *D. bolaui* reported in the present work is the highest among the previously reported values for earthworms in various world agroecosystems (table 2). Body size, surface living habits and organic rich substrate might be the causes for maximum density of *D. bolaui* worm. In a continuously reproducing population, relationship among the density of juvenile, immature and adult is of distinct vertical pyramidal type. Because of discontinuity and unstability, vertical pyramidal structure is not very much distinct.

Studies on the horizontal and vertical distribution of earthworms have been made by Dowdy (1944), Gerard (1967), Bouche (1972), Rundgren (1975), Dash and Patra (1977), Senapati *et al* (1979), Dash and Senapati (1980) and Sahu and Senapati (1986). Stratification to upper 10 cm of soil depth along with single population peak in the

pasture and two population peaks in the compost pit showed that *D. bolaui* is a surface living earthworm. Bimodality of *D. bolaui* at compost pit site might be an indication of favourable environmental factors like soil temperature, moisture and organic matter (table 1). Dash and Senapati (1980) and Senapati and Dash (1981) have reported that population survival in *Octochaetona surensis* (Michaelsen) earthworm was possible for two reasons: (i) migration of worms to a depth of > 20 cm during summer and (ii) prevalence of minimum soil moisture of about 3 g%. So the field population of *O. surensis* never became zero. But the conditions with *D. bolaui* were different in both pasture and compost pit site where the worm never migrated below 15 cm of soil depth and field soil moisture decreased to extreme values (< 1 g%) resulting in worm mortality. These could be probable reasons for zero population of *D. bolaui*.

5.2 *Reproductive strategy*

Reproductive strategy of a few species has been studied from both temperate and tropical habitats. Among the species of earthworms whose reproductive biology and life cycle have been worked out are *Eisenia foetida* (Savigny), *Allolobophora longa*, Ude, *A. caliginosa* (Savigny), *Lumbricus terrestris*, Linnaeus, *L. rubellus*, Hoffmeister, *Bimastos zeteki* (Smith and Gittins), *Eudrilus euginiae* (Kinberg), *Milsonia anomala*, Omedo, *Drawida calebi* Gates, *D. willsi*, Michaelsen, *Lampito mauritti* Kinberg and *Ochtochaetona surensis* (Michon 1954; Avel 1959; Murchie 1960; Satchell 1967; lavelle 1977, 1978; Hartenstein *et al* 1979; Senapati *et al* 1979; Dash and Senapati 1980; Senapati 1980; Pani 1986). Discontinuous mode of reproduction has been advocated for surface living earthworms (Satchell 1967; Rundgren 1977; Dash and Senapati 1980). Previous studies on tropical earthworms (*D. calebi*, *L. mauritii* and *O. surensis*) have revealed single peak and prolonged emergence pattern, whereas *D. bolaui* showed a single and sudden emergence pattern in the pasture. A double population peak following sudden emergence has been observed from the compost pit. Among the tropical earthworms known, the incubation period is about 7 days in *D. bolaui*, 15 days in *D. willsi* and 30 days for *M. anomala*, *D. calebi*, *L. mauritii* and *O. surensis*. A life cycle of about 12–20 months have been reported for *D. calebi*, *L. mauritii*, *O. surensis* and *M. anomala*. *D. bolaui* exhibited a very brief life cycle of about 4–5 months in pasture ecosystem whereas it was around 4–8 months in the compost pit site. Population survival through cocoons had been advocated by Wilcke (1952) and Murchie (1960) and has been reported for the first time in *D. bolaui*. This is an unique attribute of its epigeic (surface living) nature. Intraspecific variation of reproductive strategy with change in the habitat conditions indicate a flexibility. Thus *D. bolaui* having an adult size measuring about 4 cm in length and weighing 7 mg dry weight, with epigeic habit, high population density, reproductive rate and environmental resistance, comparatively short life cycle and life span period indicate the possibility of utilising this earthworm in biotechnology particularly in vermicomposting and also vermiculture. Comparative role of *D. bolaui* worms in the decomposition of various organic wastes are in progress. This type of study will be helpful in developing vermitechnology programme and also in the management of the decomposer fauna like earthworms in tropical agroecosystems.

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Histological and histochemical observations on the digestive gland of *Melanoides tuberculatus* (Gastropoda) infected with certain larval trematodes and focus on their mode of nutrition

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Abstract. Histological and histochemical observations were made on infected and uninfected digestive glands of snails, *Melanoides tuberculatus*. Infected gland either with sporocysts of *Cercaria diglandulata* (Xiphidio) or with rediae of *Cercaria martini* (Monostome) revealed basically two types of histopathological abnormalities (damages), mechanical (lesion 1) and physiological (lesion 2). Depletion of carbohydrates, lipids and proteins as well as increased activities of phosphatases, lipase, non-specific esterase and glucose-6-phosphatase were also reflected by infected digestive gland. Alterations either histological or histochemical in infected glands are associated with the type and size of trematode larvae and degree of parasitemea and these were greater in glands infected with rediae than in sporocysts. Besides pathogenesis, the present study also focusses on the mode of nutrition in certain trematode larvae.

Keywords. Histopathology; digestive glands; *Melanoides tuberculatus*; Parasitemea; *Cercaria diglandulata*; *Cercaria martini*.

1. Introduction

Larval trematodes, in general, cause great harm to their hosts. They multiply in snail hosts resulting in tissue damages, and the pathological changes range from debilitation to gigantism of the hosts (Rothschild 1936, 1941; Wilson and Dension 1980). Histopathological changes in molluscs associated with larval trematode infections have been described or reviewed by several workers (Cheng and Snyder 1962; James 1965; Patnaik and Ray 1966; Wright 1966; Erasmus 1972; Moore and Halton 1973; Mohandas 1974; Yoshino 1976; Sommerville 1978; Krishna 1979; Bertman 1980). Further, histochemical changes in parasitised digestive glands have also been investigated (Cheng 1962, 1963a, b, c, 1965; James and Bowers 1967a, b; Southgate 1970; Reader 1971; Gress and Cheng 1973; Michelson and Dubois 1973; Dennis *et al* 1974; Anteson and William 1975; Krishna 1980; Yadav 1981; Choubisa 1985).

In the present study, besides histopathology of the snail tissues, histochemical localization of phosphatase, lipase, non-specific esterase, glucose-6-phosphatase, carbohydrates, lipid and proteins has also been conducted in order to determine the biochemical changes in the host tissues subsequent to infection. It is hoped this study will help in revealing the loss of macromolecules, drained by larval digeneans as nutrients, from the host tissues. The study also highlights the differences in host pathology due to sporocyst, redial and cercarial infections.

2. Materials and methods

The snails, *Melanoides tuberculatus*, were collected from freshwater habitats of Udaipur, Rajasthan. The methods for rearing of snails and collection of larval trematodes were described earlier (Choubisa and Sharma 1983, 1986). For histochemical localization of enzymes and non-enzymes the methods followed were those by Pearse (1968, 1972) and Holt and Withers (1952). Paraffin sections were cut at 7 μm while frozen sections were cut at 10 μm. Controls were run simultaneously.

3. Results

Of 300 *M. tuberculatus*, 54 were infected with *Cercaria martini* (Pandey and Agrawal 1977) and 24 with *Cercaria diglandulata* (Pandey 1967). *C. martini* was located (mostly towards) in the peripherial region whereas *C. diglandulata* was found between and around the digestive tubules. However, the localization of these species was not constant.

3.1 *Morphology and histology of uninfected digestive glands*

The uninfected digestive glands of *M. tuberculatus* occupied the maximum part of the visceral mass. Brown-green in colour, it consists of two main compact lobes, each opening into the stomach through a main duct. The entire visceral mass was enclosed in thin membrane, the tunica propria.

The main histological feature was the mass of digestive tubules embedded in their stroma of fine connective tissues (figure 1). These tubules consist of a single layer of epithelial cells on a prominent basement membrane. The epithelial cells were basically of 3 types—the more frequently observed were the digestive/absorptive cells—columnar, ciliated and with a basal nucleus and numerous globules. The second type of cells (secretory cells), was oval or triangular-shaped with prominent nuclei. Some tubules showed a greater number of cells than others. The third type of cells (starving, undifferentiated cells) was seen only in the intestinal component of the digestive epithelium.

3.2 *Morphology and histology of parasitized digestive glands*

The infected digestive glands of *M. tuberculatus* either in *C. martini* or in *C. diglandulata* appeared brown or grey in colour, swollen and friable. The tunica propria appeared irregular or ruptured.

Generally, areas of inter-tubular connective tissues were occupied by parthenitae. Basically two types of damages, mechanical (lesion 1) and physiological (lesion 2) were observed. In mechanical damages (lesion 1), degenerating changes such as (i) rupture of tunica propria, (ii) reduction in diameter and number and irregular shape of tubules and consequently enlargement of inter-tubular areas, (iii) decrease in height of columnar cells and simultaneously, increase of volume of intra-tubular areas or gut lumina (figures 1 and 2) and (iv) complete or partial destruction and blocking of individual tubules (figure 3) were confined to individual tubules. Neighbouring tubules remained unaffected (figures 2 and 3, indicated by L1) and
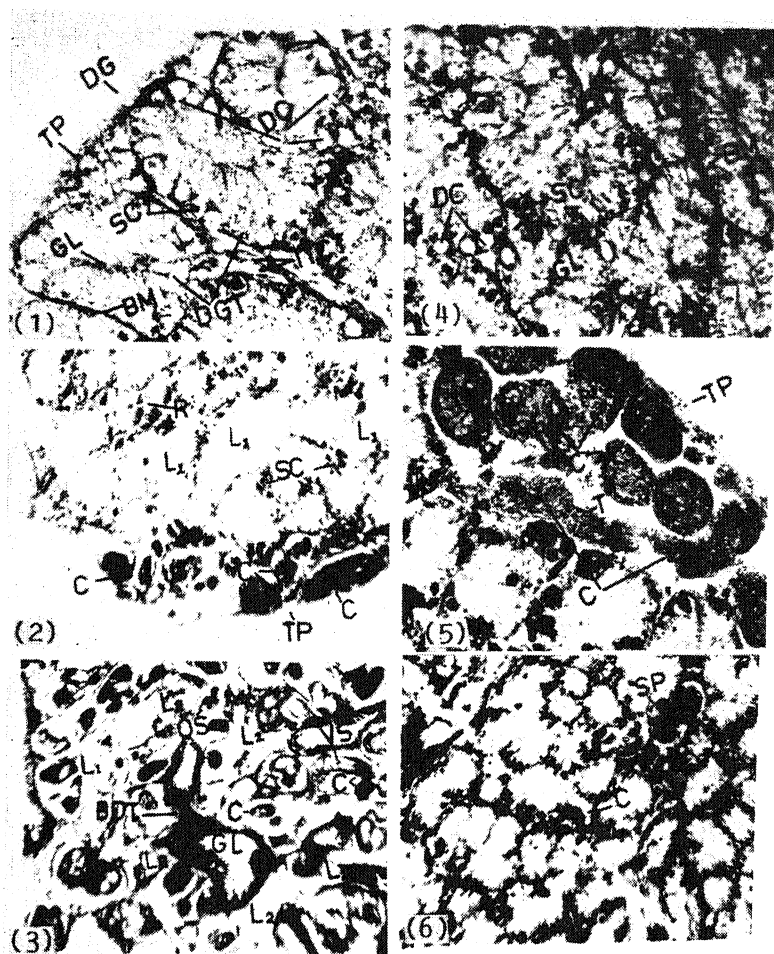
Figures 1–6. **1.** Histology of healthy digestive gland of *M. tuberculatus* stained with haematoxylin and eosin. **2 and 3.** Histology of infected digestive gland with *C. martini* (2) and *C. diglandulata* (3). **4–6.** Sections of uninfected (4) and infected digestive glands with *C. martini* (5) and *C. diglandulata* (6) showing glycogen concentration (× 240).

were observed in mild infection. In physiological damages (lesion 2) cellular organization or integrity was completely lost and necrosis of epithelial cells in heavily infected digestive gland was observed.

These pathological changes were not revealed in all cases, and depended on the degree of parasitemea and were associated more with redial of *C. martini* than with sporocysts of *C. diglandulata*.

3.3 *Comparative histochemical observations*

The digestive and secretory cells of the healthy digestive gland showed positive reaction to Best's carmine (figure 4) and a negative reaction to this stain following diastase digestion indicating the presence of glycogen. The distal border of the
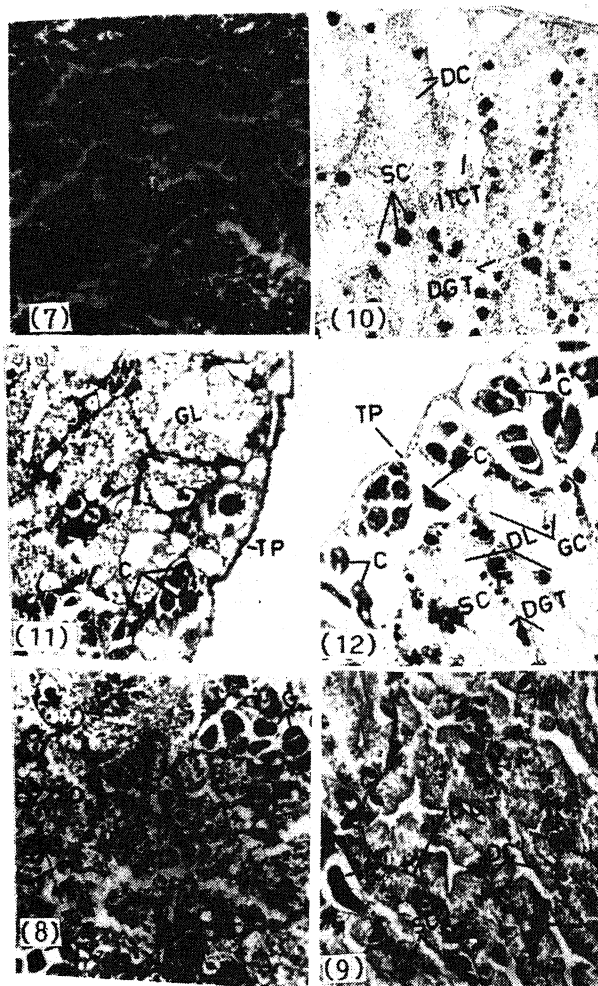
Figures 7–12. Histochemical demonstration of glycoprotein and glucose concentration in the sections of uninfected (**7, 10**) and infected digestive glands of snail host with redia of *C. martini* (**8, 11**) and sporocyst of *C. diglandulata* (**9, 12**) (× 120).

digestive cells stained deep purple red with the PAS reaction, even when pretreated with diastase. This was due to the presence of glycoprotein (figure 7). A positive reaction to Okamoto technique in the epithelial cells and inter-tubular areas of healthy digestive glands revealed the presence of glucose (figure 10) and its activity was higher in the digestive gland infected with sporocysts of *C. diglandulata* (figure 12). The intertubular spaces of heavily infected digestive gland with rediae of *C. martini* revealed greater deposition of the glycogen. Since the breakdown of digestive gland cells by rediae and the subsequent release of stored glycogen.

Strong positive reaction to Sudan black B and Oil red O in the epithelial cells of healthy digestive glands, revealed the presence of heavy concentrations of fatty acids (figure 16) and neutral fats (figure 19) but secretory cells possessed large quantities of fatty acids instead of neutral fats. These lipids were concentrated mainly in the basal
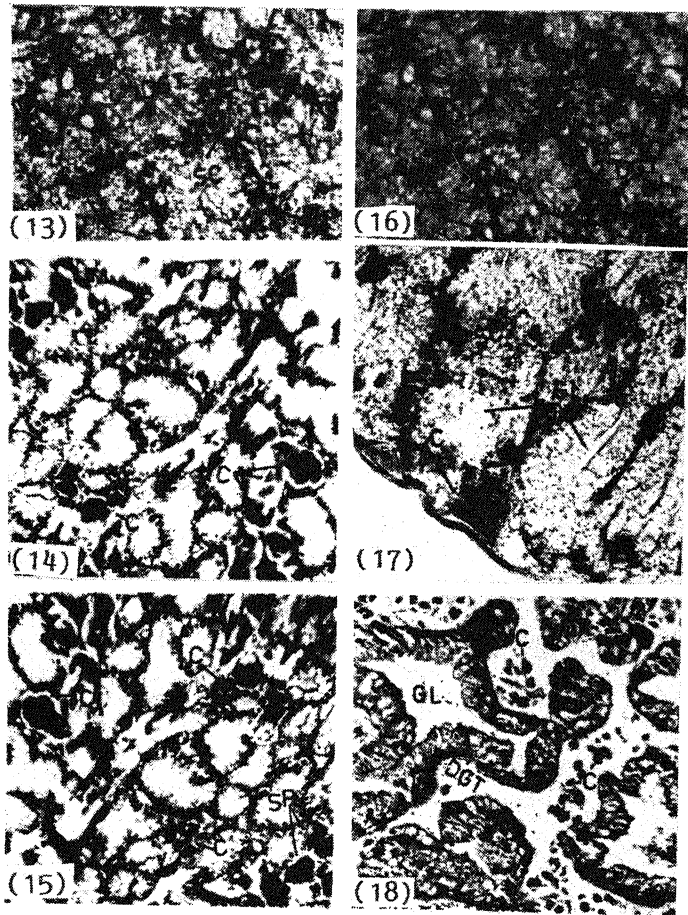
Figures 13–18. Demonstration of protein and lipid concentration in the sections of uninfected (**13, 16**) and infected digestive glands with *C. martini* (**14, 17**) and *C. diglandulata* (**15, 18**) (× 240).

regions of the epithelial cells. However, globules of fatty acids were also seen scattered throughout the inter-tubular spaces.

In general, depletion of glycogen (figures 5 and 6), protein (figures 14 and 15) and lipids (figures 17 and 18) was observed in parasitised digestive gland of hosts but depletion was maximum in those glands infected with rediae of *C. martini* (figures 5, 14 and 17).

Increased activity of lipase (figures 23 and 24), acid and alkaline phosphatases (figures 26, 27, 29 and 30), non-specific esterase (figures 26 and 27) and glucose-6-phosphatase (figures 29 and 30) was found in epithelial cells and inter-tubular spaces of infected digestive glands (table 1) and the activity of these enzymes was also proportional to the degree of parasitemea. The activity of alkaline phosphatase was greater compared to acid phosphatase (figures 26 and 27) in the parasitised digestive glands.

Simultaneously, the distribution (activity) of certain enzymatic and non-enzymatic substances in various structures of the present experimental trematode larvae has also been studied (table 2) to focus on their mode of nutrition.
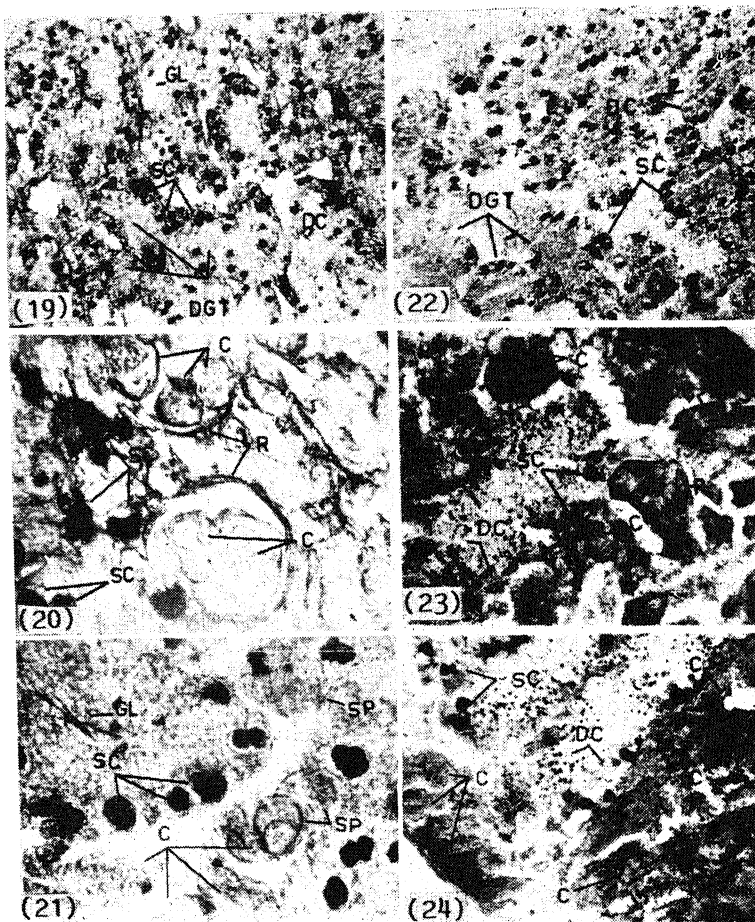
Figures 19–24. Histochemical demonstration of neutral fat concentration and localization of lipase in the sections of uninfected (**19, 22**) and infected digestive glands with *C. martini* (**20, 23**) and *C. diglandulata* (**21, 24**) (× 240).

4. Discussion

The mechanical damages (lesion 1) appear to have a cumulative effect due to larval migration, feeding and multiplication (parasitemea) as well as size and types of larvae whereas physiological damages (lesion 2) were the result of the release of proteolytic enzymes from the ruptured digestive cells/or enzymatic secretion and metabolic excretion from parasites. Starvation autolysis was due to squeezing of digestive tubules at different points and consequently no food was able to pass into the tubules. The common pathological changes like blocking of digestive tubules and reduction in height of columnar cells of infected digestive glands with either cercarial species may be due to (i) intra-tubular digestion during starvation in particulate feeders e.g. *M. tuberculatus* and (ii) reduction in amount of storage nutrients due to increasing demands of developing host gonads. The former view gets support from the work of Rees (1931) and Mohandas (1974) and the latter from Bertman (1980).
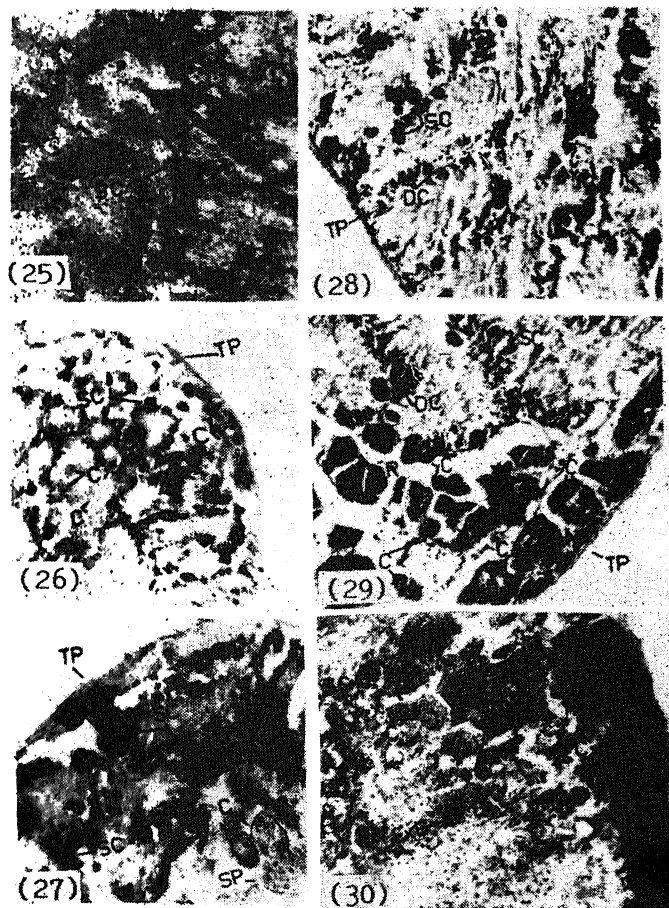
Figures 25–30. Localization of acid and alkaline phosphatase in the sections of uninfected (**25, 28**) and infected digestive glands with *C. martini* (**26, 29**) and *C. diglandulata* (**27, 30**) (× 240).

The depletion of glycogen from the digestive gland cells and its appearance in *C. martini* and *C. diglandulata* revealed that these larval digeneans utilized the host glycogen. However, its absence in the cercarial caeca indicates a different mode of procurement, probably through general body surface. This speculation lends support from the fact that a number of hydrolytic enzymes are present on the body wall for the transport of simple sugars, and that autoradiography has proved this mode of nutrition in other cercariae. The tail of *C. martini* showed maximum concentration of glycogen as compared to tail of *C. diglandulata*. The former species is an active swimmer and hence requires greater energy during its free swimming phase. The heavy concentration of glycogen in the posterior region of cercarial body was probably associated with the genital rudiments and such a situation has also been observed in other trematode larvae (Ginetsinskaya 1960; Reader 1971). Prior to absorption, the glycogen is converted to glucose and hence increased glucose concentration was found in the digestive glands infected with sporocysts.

Large sizes of glycogen molecules prevent them from absorption directly through

Table 1. Results of the histochemical tests performed on the infected and uninfected digestive glands of *M. tuberculatus*.

Histochemical test	Chemical	Digestive glands	
		Uninfected	Infected
Best's Carmine	Glycogen	+ + +	+
PAS	Glycoprotein	+ + +	+
Okamoto	Glucose	+	+ +
Sudan black B	Lipid (fatty acids)	+ + +	+
Oil red O	Neutral fats	+ + +	+
Bromophenol blue	Protein	+ + +	+
Tween 80	Lipase	+	+ +
Lead nitrate	Acid phosphatase	+	+ +
Calcium cobalt	Alkaline phosphatase	+	+ + +
Indoxylacetate	Non-specific esterase	+	+ +
Conversion of barium salt to pot. salt.	Glucose-6-phosphatase	+	+ +

+ + +, Heavy reaction; + +, moderate reaction; +, weak reaction.

Table 2. Distribution pattern of certain enzymes and non-enzymatic substances in larval forms.

Chemicals	Sporocyst (C. diglandulata)				Redia (C. martini)						
	G	B	B	W	G	B	B	W	O	G	G(C)
Glycogen		−	+ + +			−	+ + +			−	+ + +
Glucose	+ + +		+		+ + +		+			−	+ +
Lipids (fatty acid)		−	+ + +			−	+			−	+ + +
Neutral fats		−	+			−	+			−	+
Protein	+ + +		+ + +		+ + +		+ + +		·+		+ + +
Lipase		−	+			−	−		+ + +		+ +
Acid phosphatase		−	+ + +			−	+		+ + +		+ + +
Alkaline phosphatase		−	+ + +			−	+		+ + +		+ + +

Chemicals	Cercaria (both species)							
	(Immature)				(Mature)			
	S	G(C)	BS(PC)	T	S	G(C)	BS(PC)	T
Glycogen	+	−	+ +	+	+ + +	−	+ + +	+ + +
Glucose	−	−	+ + +	−	−	−	−	−
Lipids (fatty acids)	+	−	+ +	−	+ +	−	+ + +	−
Neutral fats	−	−	+ +	−	+	−	+ + +	−
Protein	+ +	−	+ + +	+ +	+ + +	−	+ + +	+ + +
Lipase	−	−	+ +	−	−	−	+ + +	−
Acid phosphatase	+	+	+	−	+	+	+ +	−
Alkaline phosphatase	+	+	+	−	+	−	+ +	−

+ + +, Intense activity; + +, moderate activity; +, low activity; −, no activity; BS(PC), body surface (parenchymatous cells); BW, bodywall; G(C), gut (Caeca); GB, germ balls; OG, Oesophageal gland; S, suckers; T, tail.

the body wall of sporocyst. Glycogen in the digestive glands of the hosts is therefore to be hydrolysed to monosaccharides, before it can be utilized by the larvae. The appearance of glucose in the inter-tubular areas of the digestive gland, in the
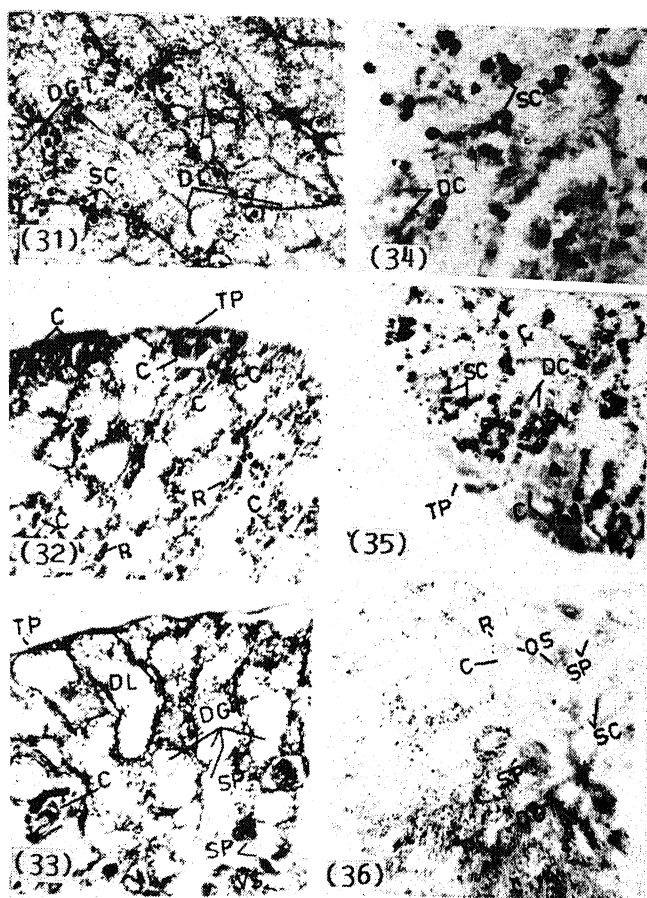
Figures 31–36. Localization of non-specific esterase and glucose-6-phosphatase in the sections of uninfected (**31, 34**) and infected digestive glands with *C. martini* (**32, 35**) and *C. diglandulata* (**33, 36**) (× 240).

(Abbreviations used: BDT, Blocking of digestive tubules; BM, basement membrane; C, cercaria; DC, digestive cells; DG, digestive gland; DGT, digestive gland tubules; ExB, excretory bladder; GL, gland lumen; ITCT, inter-tubular connective tissue; L1, lesion 1; L2, lesion 2; OS, oral sucker; R, redia; SC, secretory cells; SP, sporocysts; T, tail; TP, tunica propria; VS, ventral sucker).

sporocyst's body wall and brood chambers of *C. diglandulata*, and at the body surface of developing or immature cercariae within these sporocysts strongly suggests the absorption of the glycogen by parasites through body wall. However, the maximum depletion of glycogen in intact gland tubules infected with rediae of *C. martini* also suggests that carbohydrates may directly affect ingestion of host cells by rediae. Depletion of glycogen in the infected digestive glands has also been observed by many workers (Cheng 1963a; Reader 1974; Karyakarte and Yadav 1976; Krishna and Simha 1977).

Enhanced activity of phosphatases in infected tissues of snail appears to be the result of parasitism and varies with the intensity of infection. Such observations have also been reported by Cheng and Snyder (1962), Cheng (1964), Reader (1971),

Michelson and Dubois (1973) and Dennis *et al* (1974) in parasitised digestive gland of various molluscan species.

Redial gut and pharynx revealed higher concentration of phosphatase activity compared to their body wall. This observation supports the finding of Probert (1966), Reader (1971) and Sharma and Choubisa (1985) in the rediae of *Echinoparyphium*, *C. imbricata* and *C. tewarii* (Choubisa and Sharma 1985) respectively. In view of the presence of strong phosphatase activity on the wall of sporocysts, rediae and developing cercariae, it may be associated with the transport of glucose and other nutrients needed by the parasite (James and Bowers 1967). The appearance of phosphatases in the excretory system (Cheng 1962; Probert 1966; Reader 1971; Krishna 1980; Sharma and Choubisa 1985) may be involved in the selective resorption and energy transfer.

In infections with sporocysts of *C. diglandulata*, there was definite decrease in the neutral fat contents of digestive gland cells and this suggests that the larvae utilized the host lipids. These observations contradict those of Cheng and Snyder (1962), Cheng (1965), James and Bowers (1967b), Southgate (1970) and Krishna (1980) who reported an increase in the neutral fat content of digestive gland cells of infected molluscs with larval trematodes.

The presence of fatty acids in the body wall of sporocysts of *C. diglandulata* may indicate the passage of these metabolites from their surroundings for the ultimate use of the growing cercariae. Such fatty acids removed from the surroundings may be replaced by the degradation of neutral fat within the digestive gland. This would account for the observed decrease in the neutral fat content of infected digestive gland cells. Presumably, any fat produced by the degeneration of digestive gland cells is immediately utilized by the sporocysts, since there was no apparent increase in the neutral fat content of those tubules showing histolysis. These findings confirm the observations of Reader (1971) in mollusc, *B. tentaculata* infected with sporocysts of *C. helvelica* XII. Since the body wall of the sporocysts in the present investigation has shown the presence of lipase activity histochemically, it may be surmised that the lipase of intrinsic origin hydrolyze the host lipids into its simplest constituents to be made available to the parasite. Cheng (1965), Reader (1971) and Sharma and Choubisa (1985) have also recorded the lipase activity in the larval trematodes. The possibility of lipase to cercarial origin (cercariae inside the sporocyst) in the body and in the haemocoelomic spaces of the snail host could not be ruled out.

The fatty acids have been reported to be concentrated at the body surface of many cercariae and therefore, they may be used either in the synthesis of neutral lipids or as a source of energy for growth and development. However, fat does not appear on the surface of *C. diglandulata* until a late stage of development and this observation agrees with Ginetsinskaya (1960) and Reader (1971) on other Xiphidio cercariae. During the aerobic and free swimming phase of cercariae it appears that the fat may be used as a source of energy increasing the buoyancy of the cercariae.

Parasitised digestive glands, either with sporocysts of *C. diglandulata* or radiae of *C. martini* clearly revealed the decrease of glycogen, protein and lipid food components. However, the depletion of these components was comparatively greater in the digestive gland cells infected with rediae. The debris in redial gut was highly positive and this corresponds with the tissue feeding of the redial larvae. Such observations have also been reported by others.

The non-specific esterases (NSE) are believed to have their main sites of origin

within the secretory cells, since the secretory granules produced by these cells have been found to be NSE positive. The secretory granules pass to the apical region of the secretory cells from where they are discharged into the lumen. Alternatively secretory cells undergo lysis due to the presence of the digenean larvae, liberating secretory granules which then find their way into the lumen (Choubisa 1986). The increase of NSE in the infected host tissue, therefore, may be related to the presence of larval forms.

Acknowledgements

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Seasonal changes in the adrenal glands of the south Indian female gerbil, *Tatera indica cuvierii* (Waterhouse) in relation to the reproductive cycle

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Abstract. The adrenal glands of the south Indian female gerbil, *Tatera indica cuvierii* (Waterhouse) exhibited seasonal changes in their weight, histology and histochemistry during different months of the year. Seasonal changes seen in the adrenal glands also correlate with that of changes in the ovary. The cortical cells of the zona fasiculata and zona reticularis in the adrenal glands of sexually active gerbils show decrease in cell as well as nuclear sizes during quiescent period (April–July). The zona glomerulosa and medulla do not show any changes in their structure during different reproductive phases. Histochemically, the zona fasiculata and zona reticularis of the adrenal glands of the gerbils accumulate abundant lipids and carbohydrates during breeding period, when compared with quiescent females. The 'X' zone shows fluctuation and is present in the mature, pregnant and spayed females and disappears in parous and quiescent gerbils. It is inferred that a relatively unchanged secretion of mineralocorticoids, an increased or decreased secretion of glucocorticoids required to maintain the functional status of adrenal cortex could be possible in gerbils during the reproductive cycle.

Keywords. Seasonal variation; adrenal cortex; histological changes; lipid and carbohydrates; south Indian gerbil.

1. Introduction

The variation in the size of an endocrine gland, the period of activity, dormancy and the reproductive cycle are inter-related phenomena. The studies on the adrenal gland in relation to reproductive cycle among mammals and the available literature concerning the relationship between the adrenal cortex and reproductive activity of rodents are limited. Although the adrenal glands of many small mammals have been studied (Mc Keever 1959, 1964; Prakash 1964; Jain 1970, 1971; Gastone 1986a, b), comparatively not much attention has been paid to the functional changes in the histology of the adrenal gland in relation to the reproductive cycle. The south Indian gerbil, *Tatera indica cuvierii* (waterhouse) is a predominent rodent pest especially in dry land crops (Govinda Raj and Srihari 1987). Some studies dealing with natural history, food and feeding habits have been recorded by Prasad (1954a, b, 1961) and its female reproductive cycle has been studied by Govinda Raj (1984). The present investigation was undertaken to correlate the histological and histochemical changes in the adrenal gland with different phases of the reproductive cycle.

2. Materials and methods

Female gerbils used in the present study were collected from the wild population from the borders of the cultivated fields in the neighbourhood of Bangalore. The

collections extending over more than two years were made in different months of the year by digging the burrows and sometimes nets were also used to capture them. The histological and histochemical studies in the adrenal glands were studied in groups of females as follows:

(i) Immature: Young ones and juveniles (body weight 10–50 g).

(ii) Mature: Presence of 4 pairs of developed mammary glands (teats), body weight 80 g and above, vaginal orifice perforate.

(iii) Pregnant: Swollen abdomen and conspicuous mammary glands, embryos detected by palpitating the abdomen, body weight 100 g and above and vagina imperforate.

(iv) Quiescent: Mammary teats regressed, reduced body weights (90 g or less), vagina imperforate, on laprotomy uterus shows placental scars and ovary and uterus regressed.

(v) Ovariectomized: Adult gerbils collected during the breeding season (September to March) were bilaterally ovariectomized using anesthetic ether. The ovariectomized gerbils were sacrificed 15 days after the operation. Suitable controls were maintained.

After recording their body weights, gerbils (mature, pregnant, quiescent and ovariectomized) were immediately sacrificed using anesthetic ether and the adrenal glands were quickly removed and fixed in 10% neutral formalin or Bouin's fluid after recording their weights nearest to 0·2 mg in a torsion balance. After 24 h of fixation, the adrenal glands were washed, dehydrated, embedded and serial sections were cut at 6–8 μ. The sections were then stained in haematoxylin-eosin, Masson's tetrachrome and PAS stains for histological studies. The frozen sections of the adrenal glands were also cut at $-20°$C in a freezing microtome and the sections were stained for lipids with Sudan black B in 1% alcohol for histochemical observations.

The width of the cortical zones viz. zona glomerulosa, zona fasiculata and zona reticularis and the medulla were measured in the median sections of the adrenal glands using an occular micrometer at a known magnification. About 20–30 cells of each cortical zone were selected and measurements were taken at two diameters right angles to each other. The mean diameter of their nuclei were also measured using an eye piece micrometer. The cell size was determined by using the method of Barlow and Sherman (1972).

3. Results

The left adrenal gland weighs more than the right gland. The percentage of difference in weight between right and left adrenal glands in an adult gerbil is 11–13%.

Figure 1 shows the monthly variation in the weights of the adrenal glands of the female gerbil during different months of the year correlated with that of the seasonal changes in the ovary. Table 1 gives the cytological characteristics of the cortical and medullary cells during different reproductive phases. The change in the adrenal weight is correlated with that of the ovarian weight fluctuation seen during different months. The maximum weight of the adrenal glands seen during August–March i.e. during the period of breeding season and the loss in their weight during April–July
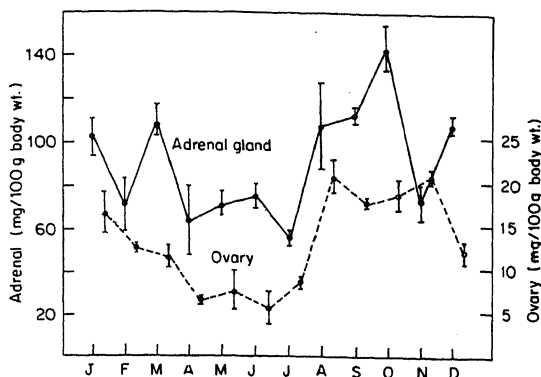
Figure 1. Monthly variation in the weight of adrenal gland and ovary. Results are mean ± SE. Bars represent SEM.

i.e. during the non-breeding season (table 2) coincides with the seasonal variation seen in the ovarian weight (figure 1).

Figure 2 shows the variation in the width of the cortical zones during different reproductive status. The broad zone of fasiculata (0·47 mm) and zona reticularis (0·38 mm) of the sexually active gerbils (figures 3–5) decrease in their width during non-breeding season. In the quiescent female (figure 6) the zona fasiculata is diminished in size (0·33 mm) followed by a decrease in the cell as well as nuclear sizes (table 1). The cells are small, nuclei are pale and the cytoplasm exhibits vacuolization (figure 7). The zona reticularis in the quiescent female is thin (0·14 mm) and pyknotic nuclei are fewer in number. The zona glomerulosa and the medulla (figure 8) do not show any change in their structure during different reproductive phases i.e. during breeding and non-breeding seasons, although there is a variation in the width of the medulla (figure 2).

Histochemically, the lipid inclusions in the adrenal glands of immature female are very sparse in the zona glomerulosa and more in the zona fasiculata. The adrenal glands of the mature gerbil during the breeding season accumulate abundant lipids in the zona fasiculata (figure 9) and almost nil in the zona reticularis. The lipids in the quiescent adrenals are sparsely distributed throughout the cortex (figure 10). The adrenals of the mature gerbil exhibit the presence of carbohydrates in the zona reticularis and zona fasiculata but totally absent in the zona glomerulosa (figure 11). During the non-breeding season, the reaction for the carbohydrates decreases in the quiescent adrenal (figure 12).

In the pregnant female, the width of the zona fasiculata is comparatively more (0·52 mm; figures 13, 14) when compared with that of normal adrenals of the mature gerbil. Histochemically, the zona fasiculata exhibits more lipid (figure 15) and the carbohydrates are abundant in the zona reticularis (figure 16). The medulla is relatively free from lipid droplets in the adrenal glands of immature, mature, quiescent and pregnant gerbils.

In the gerbil, the 'X' zone shows fluctuation and its presence is seen in the adrenal cortex of the mature (figure 17), pregnant (figure 13) and spayed gerbil (figure 19). It is absent in the adrenal cortex of the parous and quiescent gerbils leaving a small

Table 1. The morphological characteristics of the cortical and medullary cells of the adrenal gland of the female gerbil in different reproductive status.

Body wt (g) and stage	Adrenal wt (mg)	Width of the capsule (μ)	Zona glomerulosa			Zona fasiculata			Zona reticularis			'X' zone	Medulla		
			Width (mm)	Cell size (μ)	Nuclei shape	Width (mm)	Cell size (μ)	Nuclei shape	Width (mm)	Cell size (μ)	Nuclei shape		Width (mm)	Cell size (μ)	Nuclei shape
40 Pre-pubertal	22±1·25	1·25	0·04	3·25 Polygonal	0·65	0·4	8·75 Polygonal	1·30	0·33	5·00 Round	0·78		0·57 Round	6·25	0·65
125 Mature	144±10·0	0·25	0·05	6·25 Polygonal	6·65	0·47	11·30 Polygonal	5·78	0·38	6·25 Round/oval	0·52	P	0·70 Round	6·25	0·78
120 Quiescent	108±10·0	0·55	0·05	5·00 Polygonal	0·52	0·33	7·50 Polygonal	4·78	0·14	5·0 Round/flattened	0·82		0·54 Round	6·25	0·78
130 Pregnant	184±5·8	1·25	0·05	6·25 Polygonal	0·52	0·52	10·00 Polygonal	5·00	0·26	6·25 Round	0·52	P	0·66 Round	6·75	0·78
120 Ovariectomized	140±8·2	0·62	0·05	5·00 Round/polygonal	0·52	0·38	8·12 Polygonal	3·78	0·19	6·25 Round	0·52	P	0·58 Round	6·25	0·52

P, 'X' zone present.

Table 2. Changes in the adrenal weights* of the female gerbil during different months of the year.

Month	Mean weight
January	104 ± 8
February	72 ± 12
March	112 ± 8
April	64 ± 15
May	72 ± 5
June	76 ± 5
July	56 ± 6
August	108 ± 20
September	112 ± 4
October	144 ± 10
November	72 ± 5
December	108 ± 5

*Relative weights (mg). Results are the mean $\pm$ SE.
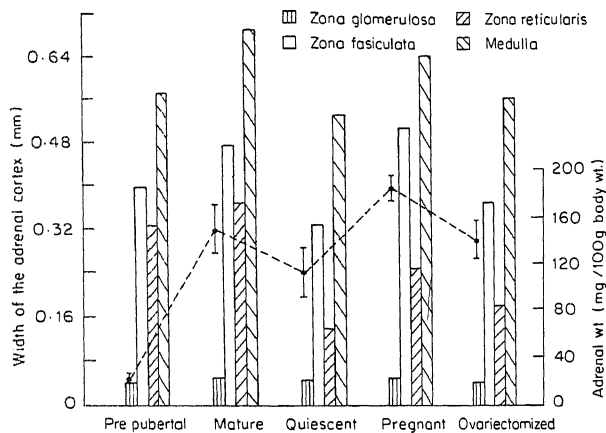
Figure 2. The weight and the width of various zones of adrenal gland of the female gerbil in different reproductive phases.

zone of connective tissue. Some of the 'X' zone cells when present contain sudanophilic inclusions (figure 9). The 'X' zone is absent in the immature gerbils.

In the adrenal glands of the ovariectomized gerbils, the zona reticularis is thin and narrow (figure 18). The cells are smaller and have pyknotic nuclei (figure 19). The 'X' zone is absent but has a small zone of connective tissue.

4. Discussion

The relative adrenal weight of the gerbil exhibits seasonal variation. The difference in the adrenal weight of the female gerbil between breeding and non-breeding period is significant ($P > 0.001$). Similar seasonal variations in the adrenal gland weights have been reported by Mc Keever (1963) in the female ground squirrel, *Citellus belding*. The glands decrease in their weight during July
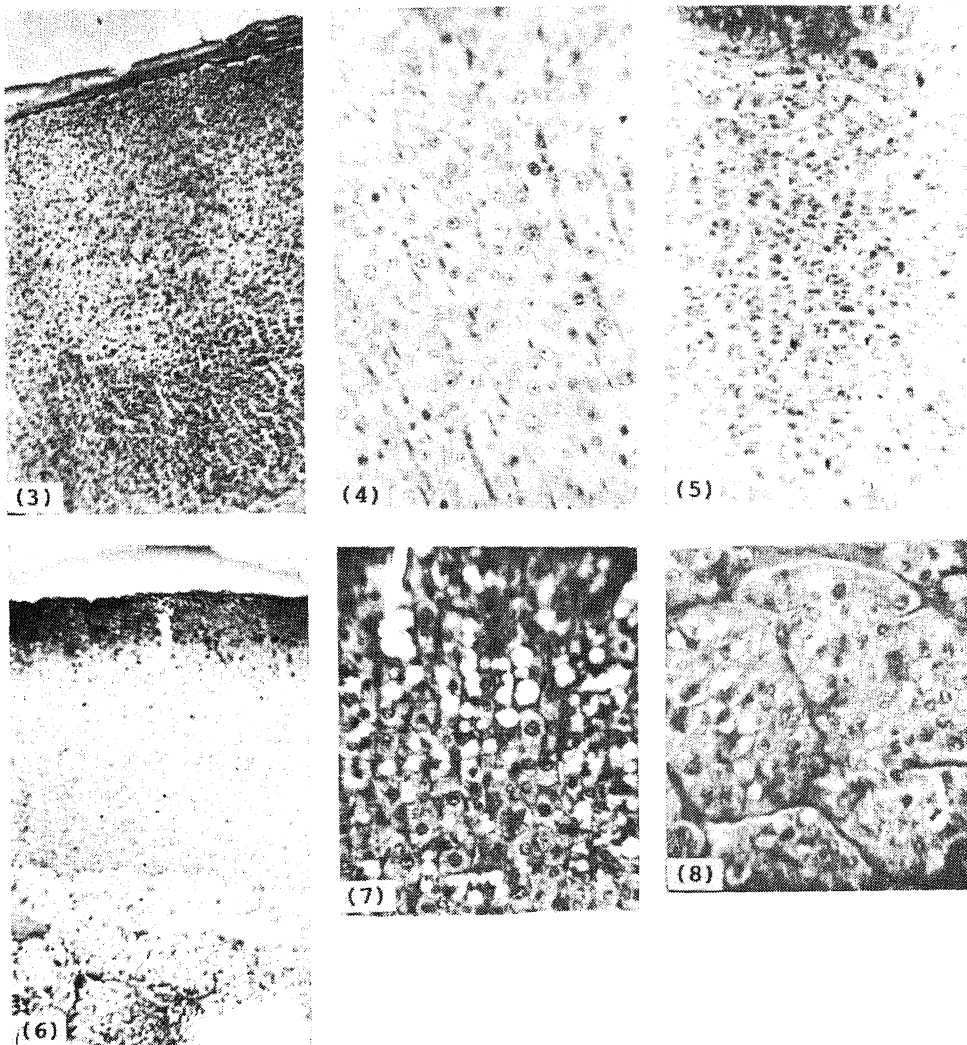
Figures 3–8. **3.** Active cortical zonation during the breeding season (H and E × 65).   **4.** High magnification of the zona fasiculata shown in figure 3, cells polygonal (H and E × 220). **5.** High magnification of the zona reticularis shown in figure 3, cells in reticulate form, with spherical to flattened nucleus (H and E × 220).   **6.** During non-breeding season showing diminished cortical zonation (H and E × 65).   **7.** High magnification of the zona fasiculata shown in figure 6, nuclei small and pale, cytoplasm exhibits vacuolization (H and E × 220).   **8.** High magnification of the medulla shown in figure 6, cells in groups, distinct nuclei, cytoplasm has few vacuoles (H and E × 220).

and August, when the animals are quiescent and aestivating. However, desert gerbils with scrotal testes had heavier adrenals than those of similar body weights but possessing regressed testes (Jain 1971). The adrenal glands of the pregnant gerbils are heavier than the lactating females. This is in agreement with the similar condition found in bank vole (Anderson and Gustafson 1980). The increase in the size of adrenal glands in pregnant gerbil, *Tatera indica cuvierii* is in contrary to the
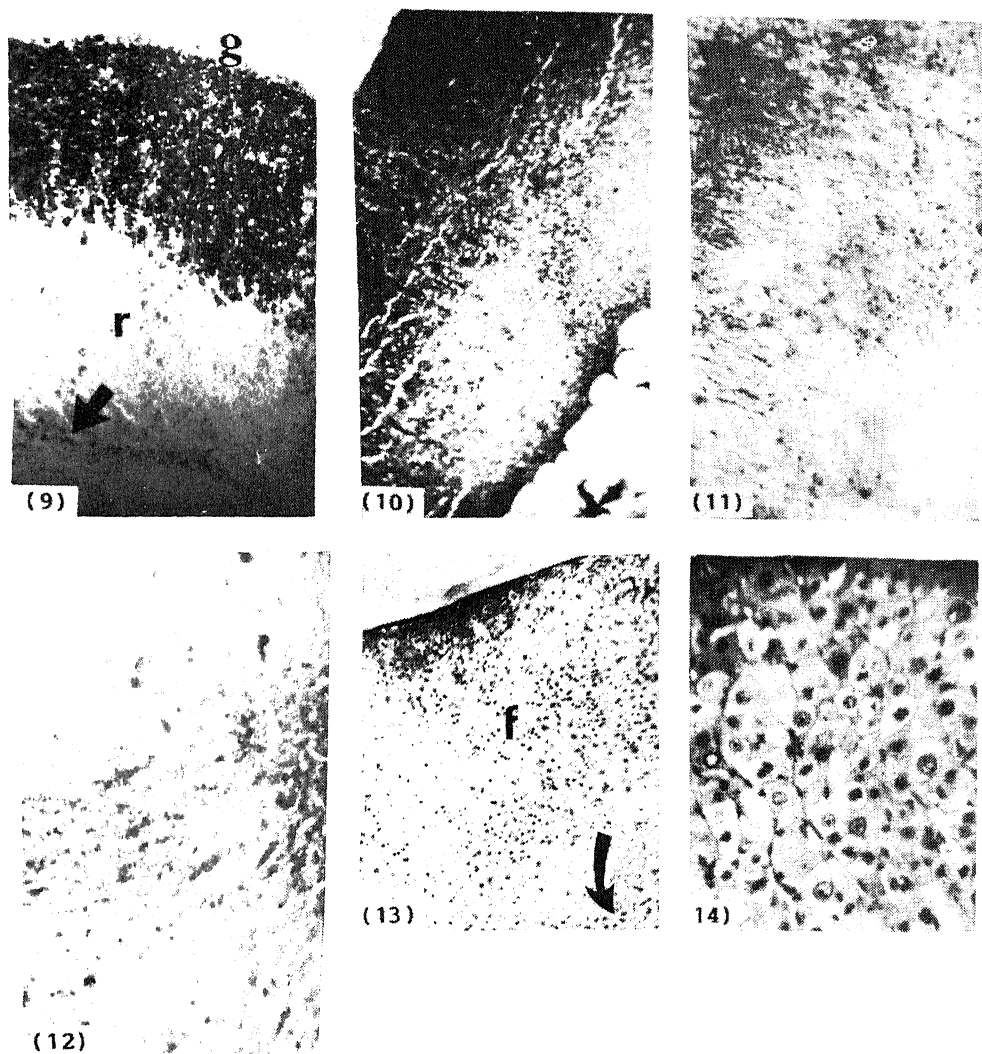
Figures 9–14. 9 and 10. The frozen sections of the adrenal gland. **9.** During breeding season—accumulation of abundant lipid only in the zona fasiculata and absent in zona glomerulosa (g) and zona reticularis (r). 'X' zone shows few sudanophilic cells (arrow). Formaldehyde calcium post chromation in dichromate calcium stained in Sudan black B (× 55). **10.** During non-breeding season-reduction of lipid inclusions in the zona fasiculata. Technique as in figure 9 (× 55). **11–13.** The adrenal cortex. **11.** During breeding season showing carbohydrates in the zona fasiculata and zona reticularis (PAS × 200). **12.** During non-breeding season showing sparse distribution of carbohydrates (PAS × 200). **13.** Pregnant gerbil showing elongated zona fasiculata (f), 'X' zone present (arrow) (H and E × 55). **14.** High magnification of the zona fasiculata shown in figure 13, cells hypertrophied (H and E × 220).

findings in the laboratory rat (Anderson and Kennedy 1933) and brown rat, *Eptesicus fussess* (Christian 1953) which showed maximum size during the period of lactation and not in pregnancy. Significant increase in the volume of
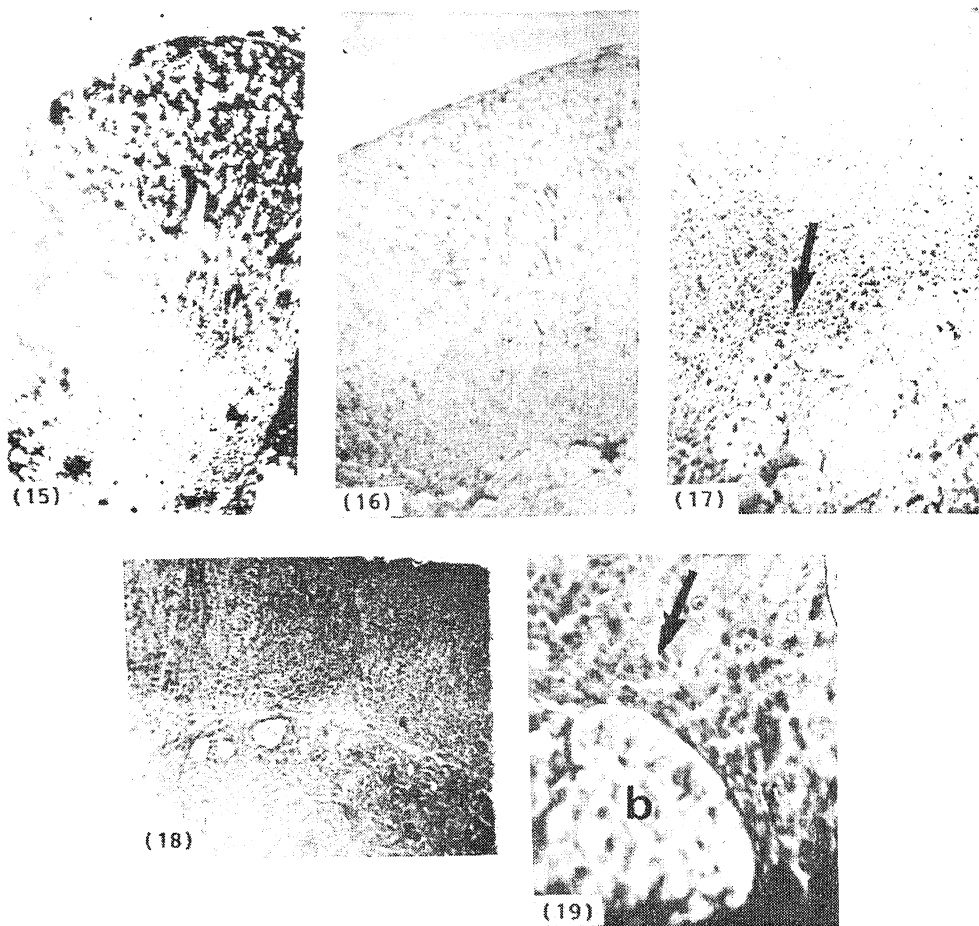
Figures 15–19. **15.** The frozen section of the pregnant adrenal-showing lipid in the zona fasiculata and sparse in zona reticularis. Technique as in figure 9 ($\times$ 55). **16–18.** The adrenal cortex. **16.** Pregnant gerbil showing abundant carbohydrates in the zona reticularis and sparse in zona fasiculata (PAS $\times$ 55). **17.** Mature gerbil-showing 'X' zone cells darkly stained (arrow) (H and E $\times$ 65). **18.** Ovariectomized gerbil showing narrowed zona reticularis. Masson's tetrachrome ($\times$ 55). **19.** High magnification of the zona reticularis shown in figure 18, cells small, nuclei pyknotic. 'X' zone present (arrow), b, blood vessel. Masson's tetrachrome ($\times$ 220).

parenchymatous cells, nuclei and lipid contents have been described morphometrically in the adrenal cortex during pregnancy (Gastone 1986a, b).

Both zona fasiculata and zona reticularis exhibited variation histologically in their width in the gerbil. During the non-breeding months of April to August, the cortical zones are narrow and less extensive. These differences in the cortical width are not consistent in various species of mammals (Zaleskey 1934; Moisier 1957; Mc Keever 1964). The adrenal cortex do not show any sign of hyperactivity in homeotherm species during summer months following diminution of ovarian function (Gastone 1986a, b). Under experimental conditions, chronic suppression (hypophysectomy and deprivation of endogenous adrenocorticotropic hormone by specific antiserum) of

hypothalamo-hypophyseal adrenal axis, induces decrease in the average volume shrinkage, signs of pyknosis leading to degeneration and death of cells in zona fasiculata and zona reticularis. The seasonal variation seen in the adrenal cortex of the gerbils may be explained on the rhythmic life processes that are compatible with the environmental factors which are prevalent with their range and ecological niche. In the south Indian female gerbil, seasonal activity is well correlated with the average growing season of food plants during the months of September to March and there is a general lack of food in the environment from April till the end of June (Prasad 1954a; Govinda Raj and Srihari 1987). To account for the cortical enlargement which is seen during the breeding season, Christian (1955, 1956) and Louch (1966) demonstrated that there is an increase in the adrenal size with an increase in the population until the population reached a point when there is a breakdown in social organization. Observations in the field mouse also confirm that adrenal weight increases with reduction in floor area up to a certain population density (Purushotham *et al* 1978).

The occurrence of lipids in the adrenal cortex is indicative of the site of storage of cholesterol esters and constitute an important precursors of steroid hormones (Deane 1958). The distribution of lipid in the adrenal gland of gerbils varies not only between the various cortical zones but also during different reproductive phases. These observations confirm the findings made in the mature desert gerbil (Prakash 1964), shrew (Balakrishna *et al* 1972) and vertebrates (Gastone 1986a, b).

The variations in the intensity of stainable glycogen and lipids seen in the adrenal cortical zones of the gerbil can be interpreted as variations in the activity of enzymes (glucose-6-phosphodehydrogenase) and consequently in the rate of steroid bio-synthesis. The secretory activity of the adrenal cortex is determined by the adeno-hypophysis (Sayers and Ronal 1975). The zona glomerulosa is the site of synthesis and secretion of aldosterone, the zona fasiculata synthesizes and secretes gluco-corticoids (Gastone *et al* 1978; Gastone 1980) and the zona reticularis is concerned with the secretion of androgenic steroids (Long 1975). In the light of these observations, a relatively unchanged secretion of mineralocorticoids, an increased secretion of gluco-corticoids could be possible in the adrenals of the south Indian female gerbil to maintain the functional status of the adrenal cortex during the breeding period of the reproductive cycle.

There have been several studies on the histology of the adrenal cortex in mammals but nevertheless there remains some lacunae concerning the variation in the expression of 'X' zone. In the south Indian gerbil, the appearance and disappearance of the 'X' zone can be correlated with reproductive condition of the female and with age (reproductive) of gerbils. In the female gerbil, the 'X' zone is present in the adrenal cortex of mature individual as in the golden hamster (Holmes 1955) but not in the immature state as in the mouse. It is also evident in the adrenal cortex of the pregnant and spayed females. The physiological significance of its occurrence is not very clear. The presence of 'X' zone in the adrenal cortex of some mammals is linked with wider problem of adreno-genital relationships (Chester Jones 1949). In the mouse, Deanesly (1928) stated that it regresses under direct or indirect influence of male hormone and thus would not support the theory that 'X' zone is particularly associated with the known androgenic activity of adrenals (Parkes 1937; Howard 1938). 'X' zone has been shown to be involved in the androgenic hormone secretion (Rass 1967; Gastone 1986a, b). The functional significance of the 'X' zone and the

probable adreno-genital relationship in the female south Indian gerbil needs further experimental study.

Acknowledgement

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Early development and implantation in the rat-tailed bat, *Rhinopoma microphyllum* (Brunnich)—Rhinopomatidae—Microchiroptera*

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Abstract. *Rhinopoma microphyllum* breeds once a year in a sharply defined season. It is monotocous, the two sides of the female genitalia being equally functional, although only one at a time. The embryo reaches the uterus as an early morula. Implantation is superficial and circumferential with the embryonic mass oriented towards the mesometrial side. The primitive amnion is formed by cavitation and the definitive amnion is formed by the development of folds. The yolk-sac develops as in all other bats. The definitive placenta is discoid, mesometrial, labyrinthine and vasodichorial.

Keywords. Early development; implantation; *Rhinopoma microphyllum*; Microchiroptera.

1. Introduction

Although for the last few decades embryologists have exhibited an increasing interest in the order Chiroptera which incorporates 19 families (Koopman 1984) yet, studies on the embryology of representatives of most families of bats are incomplete. Details concerning a single species i.e. *Rhinopoma kinneari* of the family Rhinopomatidae have been reported by Srivastava (1952), Gopalakrishna (1958) and Anand Kumar (1965).

The present work pertains to studies on the early development and implantation of *Rhinopoma microphyllum*. It was undertaken because to-date there is no record of a study on the embryology of this species which is a representative of one of the most primitive families of the sub-order Microchiroptera.

2. Material and methods

Genitalia of 186 pregnant specimens of *R. microphyllum* were collected from dungeons in dilapidated old forts and tombs at and around Burhanpur and Asirgarh (in south-western part of Madhya Pradesh). The specimens were collected at frequent intervals during the breeding season for two consecutive years with a view to ensuring that closely graded developmental stages were obtained. The female genitalia were fixed in various fixatives such as neutral formalin, Bouins, Rossman's, Carnoy's and calcium acetate formalin. Where necessary the genitalia were stored in 70% ethanol after fixation for 24 h. The tissues were dehydrated by passing through graded ethanol, cleared in xylol, embedded in paraffin and sectioned at 5–8 μm thickness. Sections were stained with Harris' haematoxylin and counterstained with eosin. Selected slides were stained by periodic acid Schiff (PAS) procedure (Pearse

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*The final work for formulating this paper was carried out at the Tinplate Hospital, Jamshedpur.

1968) some of which were counterstained with Weigert's haematoxylin. The sections were dehydrated by graded ethanol, cleared in xylol and mounted in DPX.

3. Observations

A stagewise collection summary of *R. microphyllum* is given in table 1.

3.1 *Breeding habits*

This species breeds once a year in a sharply defined breeding season. Copulation in the colony followed immediately by fertilization and pregnancy occurs in the latter half of March, and progressively advanced stages of development are available during the following weeks and the young are delivered during about the middle of July. During each cycle only one side of the female genitalia is functional without there being any dominance of either of the sides. Only one follicle reaches the pre-ovulatory phase in one of the ovaries and releases the ovum, which, after fertilization and development into a blastocyst, implants near the cranial end of the ipsilateral uterine cornu. Although both the cornua of the uterus undergo similar changes during the preimplantation stages of development of the embryo, after the implantation of the blastocyst in one uterine cornu the changes are arrested in the contralateral cornu which regresses to an anestrous condition during the rest of the gestation period. Hence, the changes occurring only in the functional uterine cornu are described in the following account.

3.2 *Free uterine stages*

3.2a *Morulae*: An early free uterine morula (figure 1) composed of 20–25 cells was located near the cranial end of the right uterine cornu. The morula had undergone a little distortion in shape during fixation and staining procedures. The zona pellucida was thick. The cells of the morula appeared to be loosely arranged with a few inter-cellular spaces in some regions of the morula. The cells had spherical lightly staining nucleus each with a distinct large centrally located nucleolus and finely granular cytoplasm.

The uterus exhibits pronounced progestational changes. The uterus had an oblong shape in transverse sections with the dorso-ventral axis longer than the lateral axis. The uterine lumen was also elongated in the mesometrial-antimesometrial axis in conformity with the shape of the uterus. The uterine epithelium was intact on all the sides and was composed of columnar cells with centrally placed slightly oblong nuclei with flaky chromatin granules dispersed in the nucleus. A darkly staining centrally located nucleolus was present in most of the nuclei. In many places the epithelium had considerably increased in height and the cells of the epithelium were closely arranged and their nuclei were located at different heights. These changes gave the uterine epithelium a stratified appearance. The uterine glands were evenly distributed on all the sides of the uterus. They were long, coiled and extended deep into the endometrium to about three-fourths the thickness of the endometrium. This region of the endometrium had become pronouncedly edematous with numerous fluid-filled intercellular spaces and widely scattered cells. Hence, this zone was lightly

Table 1. Stagewise collection details of *R. microphyllum*.

Stage of development	Specimen no. as entered in the collection diary	Date of collection	No. of specimens of a particular stage studied for the present report
Pre-ovulatory	Rh sc* 7	—	4
Graffian	Rh sc* 12	—	
follicle	Rh sc* 26		
	Rh m 107	28/3/85	
Uterine	Rh k″ 12	4/4/83	2
morula	Rh k″ 15	8/4/83	
Unilaminar	Rh k″ 14	8/4/83	4
blastocyst	Rh sc* 17	—	
	Rh m 102	28/3/85	
	Rh m 103	28/3/85	
Free bilaminar	Rh sc* 15	—	4
blastocyst	Rh m 18	19/4/83	
	Rh m 21	19/4/83	
	Rh m 24	19/4/83	
Implanting	Rh sc* 11	—	7
bilaminar	Rh sc* 14	—	
blastocyst	Rh sc* 24	—	
	Rh sc* 27	—	
	Rh k″ 13	8/4/83	
	Rh k 16	8/4/83	
	Rh m 17	11/4/83	
Implanted	Rh sc* 1	—	8
bilaminar	Rh sc* 3	—	
blastocyst	Rh sc* 6	—	
	Rh m 22	19/4/83	
	Rh m 27	20/4/83	
	Rh m 28	20/4/83	
	Rh m 30	20/4/83	
	Rh m 123	5/4/85	
Primitive	Rh sc* 20	—	7
amniotic	Rh k″ 13	8/4/83	
cavity	Rh m 22	19/4/83	
	Rh m 23	19/4/83	
	Rh m 27	20/4/83	
	Rh m 28	20/4/83	
	Rh m 30	20/4/83	
Embryonic	Rh sc* 1	—	4
disc/plate	Rh k″ 13	8/4/83	
	Rh m 19	19/4/83	
	Rh m 27	20/4/83	
Trilaminar	Rh m 19	19/4/83	2
blastocyst	Rh m 23	19/4/83	
Neural groove	Rh m 37	4/5/83	1
Allantoic	Rh m 33	4/5/83	3
diverticulum	Rh m 36	4/5/83	
	Rh m 39	4/5/83	
Early limb-bud	Rh m 35	4/5/83	1
Late limb-bud	Rh m 32	4/5/83	1
Early mid-pregnancy	Rh m 53	30/5/83	1

Table 1. (*Contd.*)

Stage of development	Specimen no. as entered in the collection diary	Date of collection	No. of specimens of a particular stage studied for the present report
Late mid-pregnancy	Rh m 41	29/5/83	1
Advanced	Rh m 40	29/5/83	4
pregnancy/full	Rh m 58	30/5/83	
term	Rh m 61	30/5/83	
	Rh m 70	19/6/83	
Full term placental disc and umbilical cord	Rh m 81	7/7/83	1

Rh sc* marked specimens are those in which the individual dates of collection were erased in the fixative due to wrong labelling.
Rh k″ are specimens which looked bigger than the rest and were thought to be representatives of a different species but were later confirmed to be the same. The verification was done by the British Natural History Museum, England.

stained. Most of the uterine glands had darkly staining columnar cells with basally situated darkly staining nuclei. The lumina of the glands contained an eosinophilic secretion. Strands of loose connective tissue separated adjacent glands and several fine maternal blood capillaries occurred in the edematous zone. A narrow zone of compact endometrium occurred between the edematous zone and the myometrial layer. In many places narrow fluid-filled spaces occurred between the deeper compact endometrial zone and the myometrium.

An interesting feature of the progestational uterine reaction in this animal relates to the fact that only one ovary releases the ovum during each cycle and the embryo is invariably implanted in the ipsilateral cornu, but both the uterine cornua exhibit equally pronounced progestational changes.

In a slightly more advanced morula which was made up approximately of 40 cells, the zona layer appeared to have become very thin, and, in some places, it had become artifactually ruptured. The nature of the cells of the morula was the same as before.

There were significant changes in the structure of the uterus. The uterine epithelium in most places had increased in height and appeared to be composed of 4–5 layers of cells due to the occurrence of the nuclei of the cells at different levels, specially in some regions where the cell walls appeared to have broken down leading to the formation of a plasmodium-like structure. In some of these regions the uterine epithelium had become separated from the underlying endometrial stromal tissue and a space had formed between the epithelium and the underlying endometrium. There was no change in the nature and distribution of the uterine glands except that the proximal ends of some of the glands seemed to have disintegrated and their cells had become merged with the endometrial stromal tissue. In such regions the endometrial stroma subjacent to the uterine epithelium appeared to be more compact than the rest of the edematous zone of the endometrium. The fundic ends of some of the glands had become enlarged into large spherical balloon-shaped structures with thin walls composed of low cubical cells. The rest of the segments of the uterine glands were composed of columnar cells, and the wall of these glands had

become collapsed towards the centre of the gland thereby reducing the width of the lumina of the glands. In all these cases the nuclei were basally situated, spherical and darkly staining. An important change in the uterine edematous zone was the pronounced increase in the vascularisation of this zone where numerous blood capillaries were present. There was no change in the deeper compact zone of the endometrium where the cells were closely crowded together and possessed dark irregularly shaped nuclei.

3.2b *Unilaminar blastocysts*: Early free unilaminar blastocysts (figures 2–4) were present near the cranial end of either the left or the right uterine cornu around the end of March. Gradually with growth they were seen in the tubo-uterine junction, in the uterine lumen (figures 5 and 6) and later in a slightly enlarged pocket of the uterine lumen (figure 7). A thick eosinophilic zona pellucida seen in the early stages thins and appears as a partially dissolved irregular mass around the embryo in slightly more advanced stages, disappearing altogether in later stages.

The inner cell mass was made up of compactly arranged cells each having granular cytoplasm and a spherical nucleus containing a darkly staining nucleolus and irregularly scattered chromatin material which, after differentiation consists of large polygonal cells. The embryonic mass with advancement is made up of a group of compactly arranged cells attached to one side of the blastocyst.

The trophoblast was made up of cuboidal cells, cubical to flat in later stages, with spherical nuclei each with a darkly staining nucleolus. The blastocyst cavity occurred on one side. The trophoblast cells lay at considerable distances from one another and were connected by thin cytoplasmic strands.

The uterine lumen was small initially slowly widening with the growth of the embryo. The uterine epithelium was intact on all sides and consisted of tall columnar cells. As the lumen widened the epithelium had become thicker and taller on all the sides of the uterus and, in most places, it presented a multilayered appearance due to the close approximation of the nuclei which lay at different heights. In many places the cell walls were not distinct in the epithelium and in most of the regions the epithelium had become separated from the underlying endometrial stroma.

The deeper segments of the uterine glands had large lumina. The gland epithelium was composed of cuboidal cells with darkly stained basally located nuclei, and their lumina contained eosinophilic secretion. The endometrium was richly vascularized and had numerous blood capillaries which appeared to be markedly enlarged near the luminal border of the endometrium. In a later stage the glands were long, tubular and coiled and opened into the uterus on all sides. They had wider lumina and the gland epithelium was composed of cuboidal cells. Immediately subjacent to the epithelium the luminal ends of many uterine glands had undergone disintegration and their cells had become merged with the cells of the endometrial stroma. However, in the deeper regions the glands were elongated, coiled and had distinct lumina, many of which, had enlarged into balloon-shaped spherical fundic ends. The gland epithelium was uniform and was composed of cubical to low columnar cells with centrally placed darkly staining nuclei. The endometrial stroma in the deeper regions was edematous. Numerous maternal blood capillaries traversed the thickness of the stroma. Thus, this part of the endometrium appeared to be richly vascularized. The endometrium at the maternal border was compact and formed a thin layer separating the myometrium from the edematous zone of endometrium.
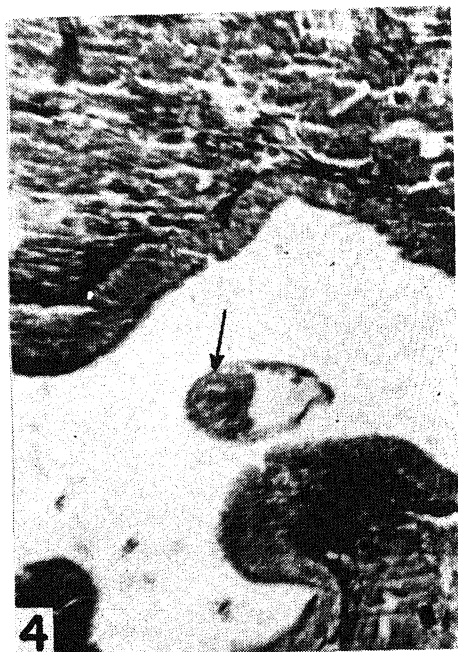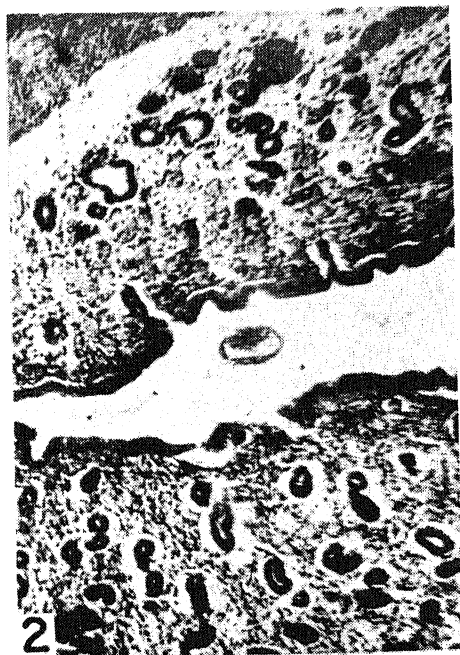
Figures 1–4. **1.** Transverse section of the uterus containing an early free uterine morula. Note the distinct zona pellucida and the presence of intercellular spaces. Uterine lumen is slit-like and the uterine epithelium is made up of columnar cells. Note the long coiled uterine glands ($\times 250$). **2 and 3.** Section of the uterus containing a free blastocyst enveloped by a distinct zona pellucida. The embryonic mass has differentiated into a bunch of cells at one pole of the blastocyst while the blastocyst cavity occurs on the other pole. Note the edematous superficial zone of endometrium containing long coiled glands and many blood capillaries (**2.** $\times 95$; **3.** $\times 250$). **4.** An advanced free unilaminar blastocyst. The zona pellucida (arrow) is thin and occurs on one side ($\times 410$).
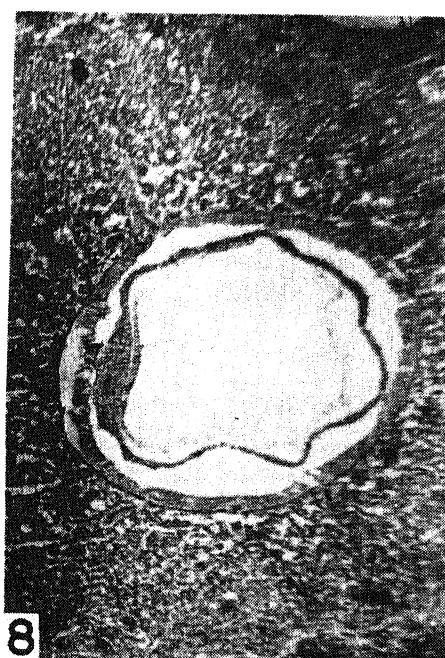
Figures 5–8. For caption, see p. 282.

3.2c   *Bilaminar blastocysts*: Seven free bilaminar blastocysts in different stages of development were observed in detail. They were found in the cranial segment of the uterus (figure 8), enlarged into a pocket in some cases (figure 9). The embryonic mass was expanded into a thick fusiform disc (spindle-shaped in sectional views) with two to three cell thickness in the centre of more advanced stages, gradually tapering towards the margins.

A few blastocysts (figures 11 and 12) had just come into contact in a few places with the uterine wall. The flat embryonic disc in one (figure 10) faced the side of the uterus which was slightly lateral to the mesometrial side while in the other (figure 12) a small amniotic cavity was present in the embryonic plate which was facing the mesometrial side of the uterus.

Endoderm had differentiated and occurred as a lining of flat cells under the trophoblastic wall of the blastocyst. The endodermal cells were cuboidal underneath the embryonic disc and were widely separated and flat (in sectional views) and were connected with one another by thin cytoplasmic strands in the rest of the blastocyst.

With the free bilaminar blastocysts lying in the uterine lumen the uterine epithelium had become tall and appeared multilayered. It was separated from the underlying endometrial stromal tissue in some places by narrow slit-like spaces. In later stages the epithelial cells in the process of disintegration had lost their cell walls and hence, their definitive shape. In the blastocysts which had just come in contact with the uterine wall the epithelium was reduced in height and attenuated. In a few places, the trophoblast had proliferated to form small conical masses which were embedded in the epithelium (figure 11).

Uterine glands and endometrium also underwent changes with the growth of the blastocysts. In the early stages the proximal segments of most glands lying subjacent to the uterine epithelium had undergone disintegration, and their cells had merged with the cells of the endometrial stroma giving a compact appearance to the zone of endometrium. Numerous blood capillaries were present in this zone. The distal segments were enlarged with large lumina bordered by low cubical cells and were at various stages of destruction. In later stages they had lost their epithelium. The coiled uterine glands occurred on all the sides. The stroma was edematous in the deeper regions of the endometrium. A thin layer of compact endometrium was present between the edematous zone and the myometrium. By the time the blastocysts were implanting the compact zone of endometrium occupied about half the thickness of the endometrium and no gland was seen in this zone. A few expanded fundic ends of the glands with flat cuboidal epithelium occurred in the

Figures 5–8.   5 and 6. Section of the uterus containing a hatched free unilaminar blastocyst. Note the occurrence of nuclei of the columnar epithelial cells at different levels giving the epithelium (arrow) a multilayered appearance. The pronounced edematous condition of the superficial zone of the endometrium is sharply demarcated from the deeper compact endometrium (5. ×95; 6. ×250). 7. Section of the uterus containing an advanced free unilaminar blastocyst. In the figure, the embryonic mass is distinct but the abembryonic part of the blastocyst appears to have torn out artifactually. Note the uterine epithelium being separated from the endometrium. Note the presence of numerous coiled glands in the superficial edematous zone of the endometrium (×95). 8. A free early bilaminar blastocyst is seen in an expanded chamber of the uterine lumen. The embryonic mass is discoid. The endodermal layer has extended on all the sides of the blastocyst. Uterine epithelium appears to be undergoing some cytolytic change. Note the compact nature of the superficial endometrium and the absence of distinct glands therein (×120).
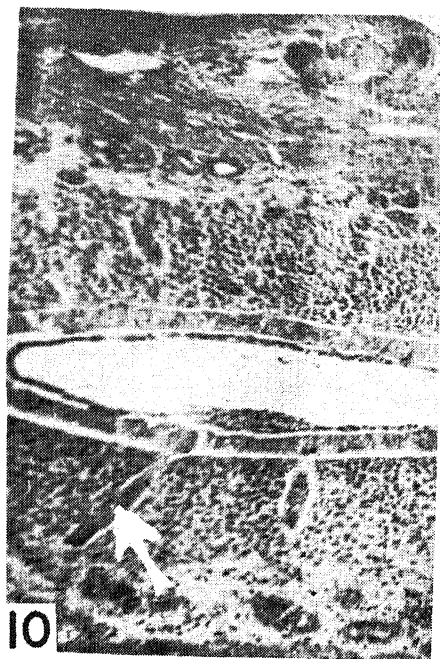
Figures 9–12. **9.** Section of the uterus containing an advanced free bilaminar blastocyst. Please see text for description ($\times 250$). **10.** Part of the section of the uterus containing an implanting blastocyst. Note some of the disintegrating glands in the superficial zone of the endometrium (white arrow) ($\times 95$). **11.** Part of figure 10 enlarged to show the proliferation of the trophoblast and its invasion into the uterine epithelium in the form of conical protuberances (white arrowhead) ($\times 250$). **12.** Part of the section of the uterus containing an implanting late bilaminar blastocyst. Note the presence of a primitive amniotic cavity (shaded arrow). Please see text for description ($\times 250$).

deeper edematous zone of the endometrium. Numerous maternal blood capillaries irrigated the compact zone of the endometrium.

3.3 *Implanted bilaminar blastocysts*

In a late bilaminar blastocyst (figure 13), which had established contact with a part of the uterine wall, the blastocyst was composed of a flat embryonic mass two to three
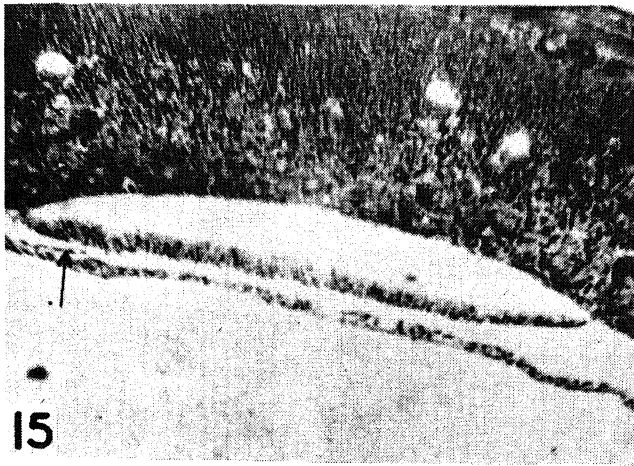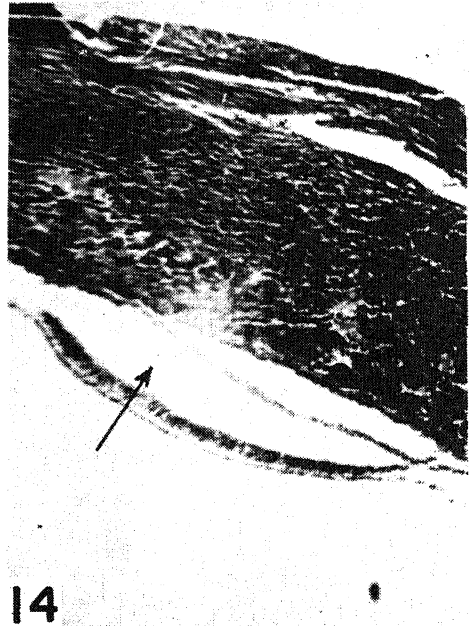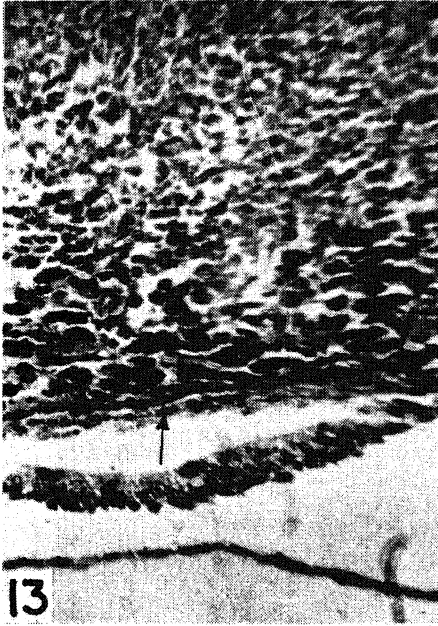
Figures 13–15. **13.** Section of the uterus containing a late implanted bilaminar blastocyst. Please see text for description. Arrow points to the thin roof of the primitive amniotic cavity ($\times$ 250). **14.** The embryonic part of an advanced bilaminar blastocyst with a large primitive amniotic cavity (arrow). Please see text for description ($\times$ 95). **15.** Embryonic part of an early trilaminar blastocyst. Please see text for description. Arrow points to the mesoderm layer beneath the embryonic plate ($\times$ 95).

cells thick within which a small space, the primitive amniotic cavity was present. The trophoblastic layer, which formed the roof of the cavity, had become stretched and occurred as a thin membrane of flat cells with small darkly staining nuclei. The embryonic plate was facing the mesometrial side and the abembryonic wall was in contact with the antimesometrial side. The endodermal layer was differentiated and formed a distinct lining to the inner surface of the embryonic mass. The endodermal cells were widely scattered underneath the trophoblastic layer on all the sides and were connected with adjacent cells with fine cytoplasmic strands.

In a later stage the blastocyst had established contact on its entire surface with the uterine wall such that there was no remnant of the uterine lumen at this level. Figure 18 schematically illustrates the morphological relationship of the blastocyst to the uterus at this stage. In a considerably more advanced stage of development the embryonic plate had expanded and there was a distinct primitive amniotic cavity within it (figure 14).

The changes in the uterus in early stages of implantation were a little more accentuated than those observed at the implanting bilaminar blastocyst stage. However, in the more advanced stages of development of implanted blastocysts the uterine epithelium had become extremely attenuated to form a very thin membrane in most places, and was altogether absent from a few places where, the trophoblastic layer was in contact with the endometrium. In a few places, where the epithelium still persisted intact, the
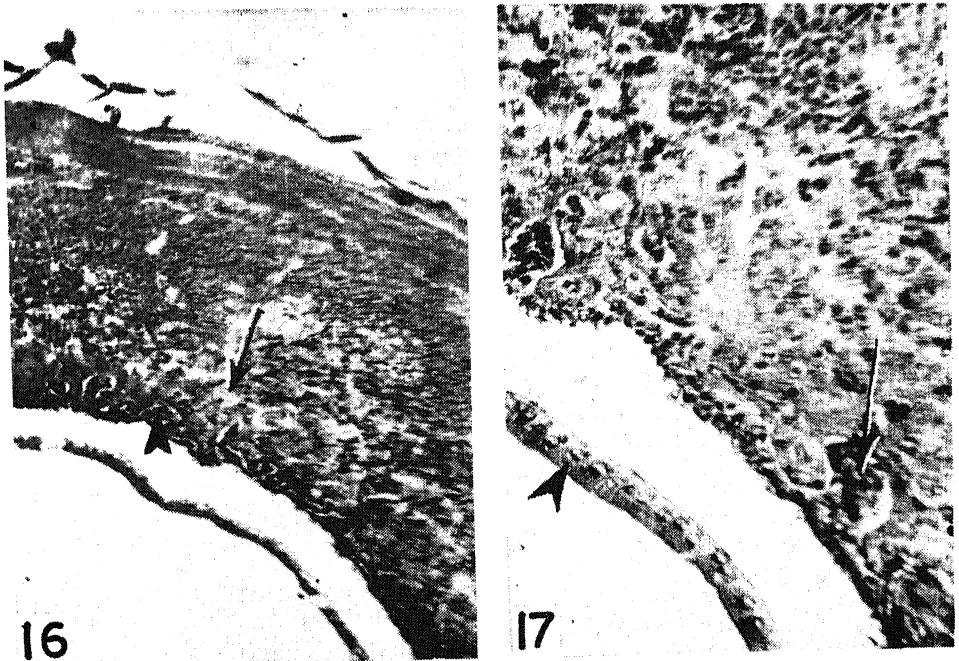
Figures 16 and 17. **16.** Lateral wall of the uterus containing the blastocyst shown in figure 15. Note the superficial zone of syncytiotrophoblast (arrow) into which the basal cytotrophoblastic layer has entered in the form of blunt projections (arrowhead) (×95). **17.** Part of the figure 16 magnified to show the syncytiotrophoblastic zone (white arrow) and the invading cytotrophoblastic projections (shaded arrow). Arrowhead points to the endodermal layer (×250).
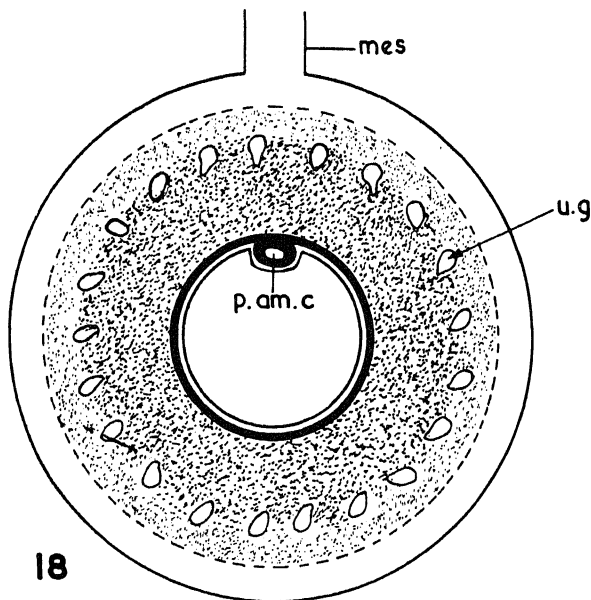
Figure 18. Semischematic drawing to illustrate the morphological relationship between the implanted blastocyst and the uterus. Please see text for description (mes, mesometrium; p.am.c, primitive amniotic cavity; u.g, uterine gland).

cells showed distinct signs of degeneration—their nuclei had become irregular and pycnotic and the cytoplasm vacuolated. The entire uterine wall at the nidation level had become compact due to the disintegration of the glands and the merger of these cells among the cells of the stroma. Only a few small fundic bulb-like remnants of glands with their wall occurred just below the myometrial layer.

3.4 *Trilaminar blastocysts*

A more advanced stage than the previous one but which is technically a trilaminar blastocyst stage had completely implanted (figure 15). The embryonic plate was wide and had expanded and formed the floor of a large primitive amniotic cavity, whose roof was formed by the superficial layer of basal cytotrophoblast since the tropho-blastic layer which had formed the original roof of the primitive amniotic cavity had disappeared. The embryonic plate was facing the mesometrial side of the uterus. Mesoderm had differentiated and occurred underneath the embryonic plate as a layer of loosely arranged cells but had not extended beyond the embryonic plate. The trophoblast had undergone proliferation and had entered the endometrial tissue and had formed a narrow zone of syncytiotrophoblast (figures 16 and 17) on all the sides of the implantation chamber. In some places the basal cytotrophoblastic layer had entered the syncytial zone in the form of numerous small solid finger shaped or club-shaped projections composed of darkly staining small cells. The deeper regions of the endometrium were compact and there were very few remnants of the fundic segments of the glands in this region.

4. Discussion

The two sides of the female genitalia are equally functional and either ovary may release an ovum in each cycle. The embryo reaches the uterus as an early morula as is also observed in most bats excepting pteropids, emballonurids and phyllostomatids (Rasweiler 1979).

The early orientation of the embryonic mass is mesometrial in *R. microphyllum* and as the embryo advances in development the embryonic mass expands into a disc which also faces the mesometrial side.

The blastocyst gets attached to the uterus on its entire surface otherwise referred to as diffuse or circumferential (Srivastava 1952; Gopalakrishna 1958).

The primitive amniotic cavity is formed within the embryonic mass in the form of a few small intercellular spaces which coalesce and enlarge into a large cavity. The roof of the primitive amniotic cavity persists and undergoes proliferation and enters the uterus. The definitive amnion is formed by the development of folds from the margins of the embryonic disc.

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Electron microscopic studies on the follicle epithelial cell—oocyte cortex interface in *Arrhenothrips ramakrishnae* Hood (Insecta: Thysanoptera)

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Abstract. Observations relating to nuclear and perinuclear-cytoplasmic changes in the follicular epithelial cells as well as changes at the follicle epithelial cell-oocyte cortex interface in *Arrhenothrips ramakrishnae* Hood, based on transmission electron microscopy are presented. The significance of increased pinocytosis during the later stages of the growth phase of follicle epithelial cells, the appearance of refractile bodies, as well as the apposition of numerous mitochondria of varied configuration to the rough endoplasmic reticulum are discussed.

Keywords. FEC-oocyte cortex interface; pinocytosis; refractile bodies; rough endoplasmic reticulum.

1. Introduction

The sequential changes involved in the differentiation and development of the oocyte in *Arrhenothrips ramakrishnae* Hood based on light microscopy, as well as studies involving the transmission electron microscope (TEM), while indicating overall similarities with the panoistic ovarian types so far investigated, have nevertheless revealed differences more particularly in relation to both structural and functional changes occurring at the follicle epithelial cell (FEC)–oocyte cortex (OC) interface (Ananthakrishnan 1988a, b). As a result of sustained influx of pinocytic elements, such cytoplasmic components as mitochondria, endoplasmic reticulum, golgi bodies, etc. tend to get displaced, becoming concentrated in a zone below the pinocytic cortex. Regarding the contribution of the FEC directly to both the yolk and cytoplasm of the oocyte, there are conflicting opinions, but the positive role of the FEC in this direction has been explained by several workers. While in general, four phases of vitellogenesis are recognized—preparatory phase, cortical vitellogenesis, completion phase and envelope formation phase (Bitsch and Bitsch 1982)—it is possible to recognize from the studies on *Arrhenothrips* as well as of other known insects with panoistic ovaries, six clear-cut stages, viz. (1) undifferentiated cortex with no pinocytosis, (2) development of interdigitating cell processes, (3) complexity of OC with development of communication channels between FEC and OC, (4) development of perivitelline or extracellular space around the oocyte, (5) more active pinocytosis and formation of cortical yolk spheres, (6) loss of FEC and oocyte contact upon chorion formation. There is therefore considerable integration between the follicle cell differentiation and the oocyte, so that both the OC and oolemma become competent to incorporate yolk precursors (Huebner 1981). Information presented here pertains to more detailed observations regarding nuclear and perinuclear-cytoplasmic changes in the FEC as well as changes at the FEC-cortex interface in the oocyte of *A. ramakrishnae* on the basis of TEM studies.

2. Materials and methods

The ovaries of *A. ramakrishnae* were dissected out in insect Ringer solution and ovarian follicles at different stages of development were fixed in 2–3% glutaraldehyde + 2–3% paraformaldehyde in 0·1 M phosphate buffer (pH 7·2). The tissue was post fixed in osmium tetroxide, rinsed in buffer, kept in 1% uranyl acetate overnight at 0–4°C and then dehydrated. Finally the material was embedded in an Epon-Araldite mixture which was polymerized at 60°C. Ultra-thin sections were cut with an ultramicrotome and examined in a TEM.

3. Results

Cellular multiplication, form changes and development of synthetic organelles are typical of the FEC. The initially small nucleus of these cells increases in volume with cell size increases and progressively changes to a more amoeboid form, occupying most of the cell. The nucleolus is large, roundish and centrally located initially, the chromatin of the nucleolus becoming dispersed into several masses, some of which are closely applied to the nuclear membrane. With further development of the FEC, the nucleus tends to become excentric. The dispersed nucleolar chromatin masses naturally contain RNA and subsequent changes in nuclear configuration followed by nipping off of small droplets or vesicles containing nucleolar material appear indicative of the role played by the nucleolar RNA in the synthesis of protein precursors, an aspect which has been confirmed in this study. It has also been observed on the basis of autoradiographic studies that follicle cell RNA is concerned with protein synthesis (Telfer 1965). The changing configuration of the nucleus, nucleolus and chromatin are indicated in figures 1–3. FEC being of the nature of actively secreting cells, numerous smooth surfaced cisternae making up the golgi complex are visible, scattered in the cytoplasm among the extensively developed rough-surfaced endoplasmic reticulum (RER), apposed to which are numerous mitochondria enabling concentration of yolk precursors (figure 6). Cortical reorganization is important since the OC is an active centre which will later become the site of the blastoderm and germ band formation, the initial cortical changes taking place during vitellogenesis, and the subsequent changes effected following vitellogenesis.

The epithelium formed by the follicle cells facilitates transmission of yolk precursors. At the end of vitellogenesis the transport becomes restricted and finally terminated. The appearance of interdigitating processes and the presence of numerous communication gaps in the vitelline membrane are indicative of their being active routes of transport (Ananthakrishnan 1988a, b) between the follicle cells and the oocyte. The enlargement of the oocyte membrane by numerous villi and folds is as typical of *Arrhenothrips* oocyte as it is of other insects with panoistic ovarioles (Bassemir 1977). The presence of an intercellular matrix in the periocytic space and in the spaces between the follicle cells has also been indicated earlier (Ananthakrishnan 1988a, b). As a result of more intense vitellogenic growth, there takes place an inward extension of the oolemma so that the follicle cell–oocyte interface undergoes structural modifications, presumably in view of increased uptake of material from the haemolymph. The FEC–oocyte interface shows several pinocytic vesicles or pinosomes (figure 4C) and, interestingly enough, during the later stages of the growth phase of FEC, the continuance of pinocytosis in the region suggests that the
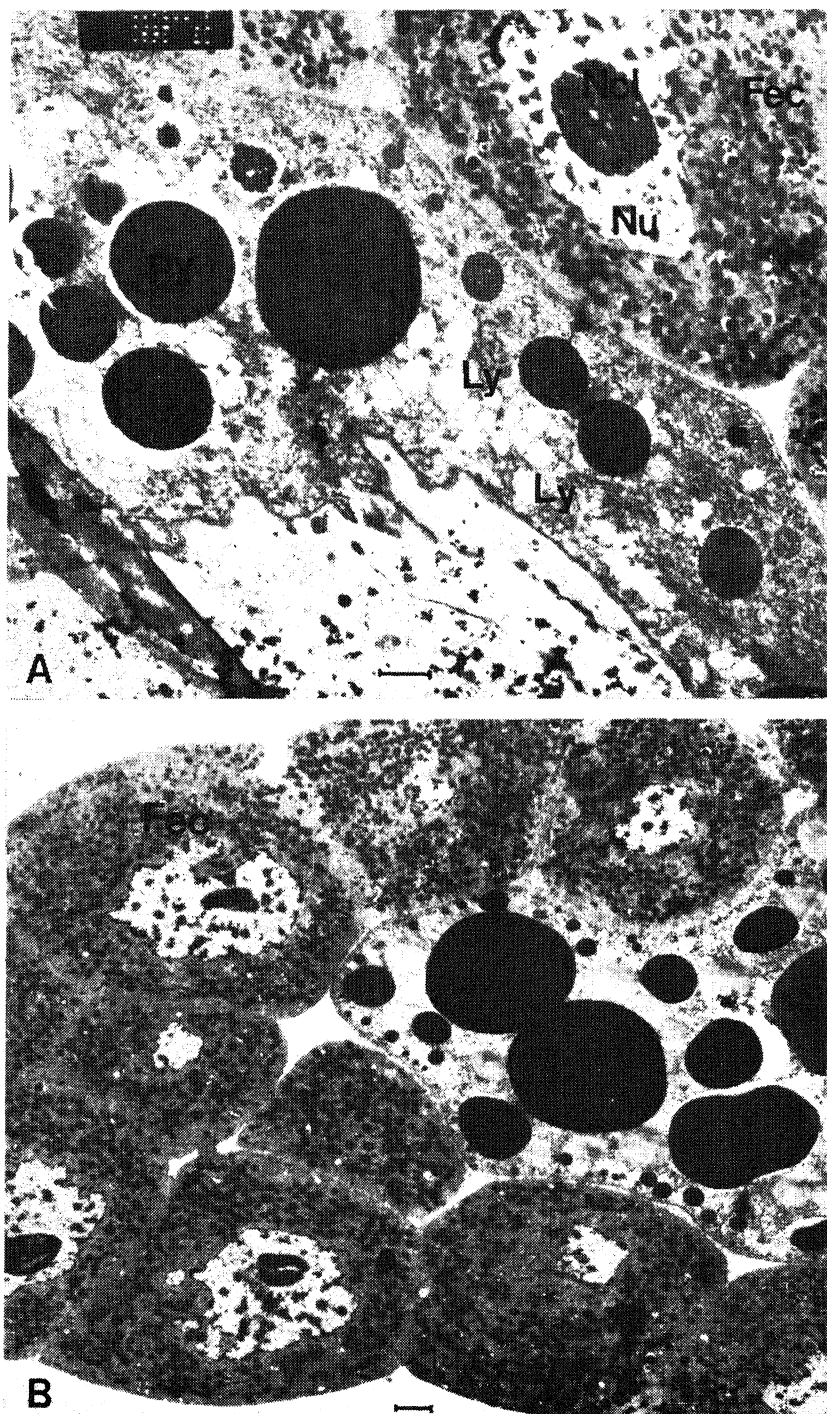
Figure 1. A. Portion of oocyte and FEC. **B.** Oocytes surrounded by many FECs.
FEC, follicular epithelial cell; Ly, lysosome; Ncl, nucleolus; Nu, nucleus; Py, protein yolk.
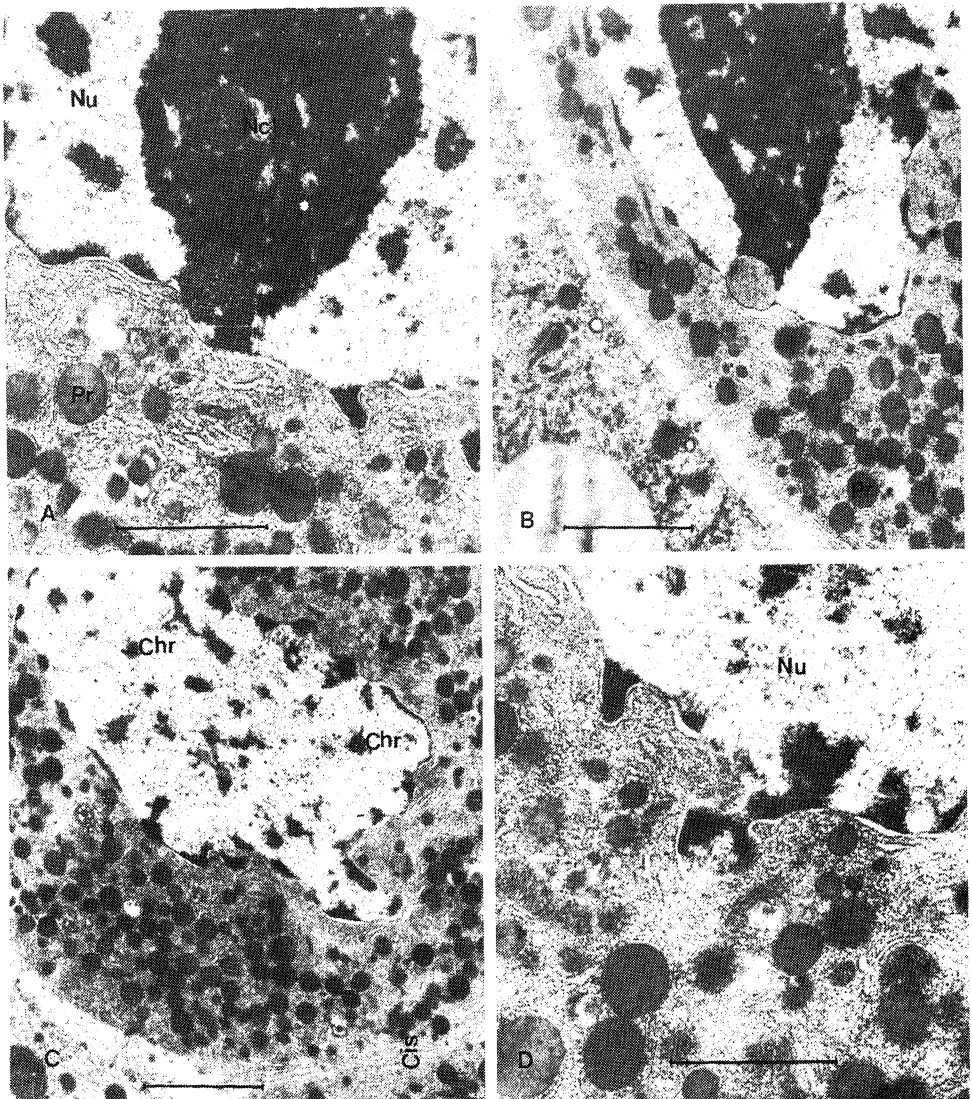
Figure 2. Sequential changes of nuclear and nucleolar material of the FEC during vitellogenesis.
Chr, chromatin; Cis, cisternae; Pr, yolk precursor.

secretion of FEC may be incorporated with the refractile bodies (figure 7) by the oocyte (Telfer and Smith 1969). With the cessation of pinocytosis in the later stages of development, the cortex does not produce protein yolk spheres, but produces transitory elements called the refractile bodies, which become vacuolated by the end of the terminal phase. Immediately below the ooplasmic microvilli small vesicles appear in the cortical cytoplasm of the oocyte. Throughout the period of yolk formation the oocyte cortex tends to maintain a high degree of vesicle formation and fusion. Alongside the vesicles a large number of membrane-bound tubules are also evident (figure 4A, B). These are presumably the residues of vesicles which have emptied their contents into the yolk spheres (Telfer and Smith 1969). To be precise,
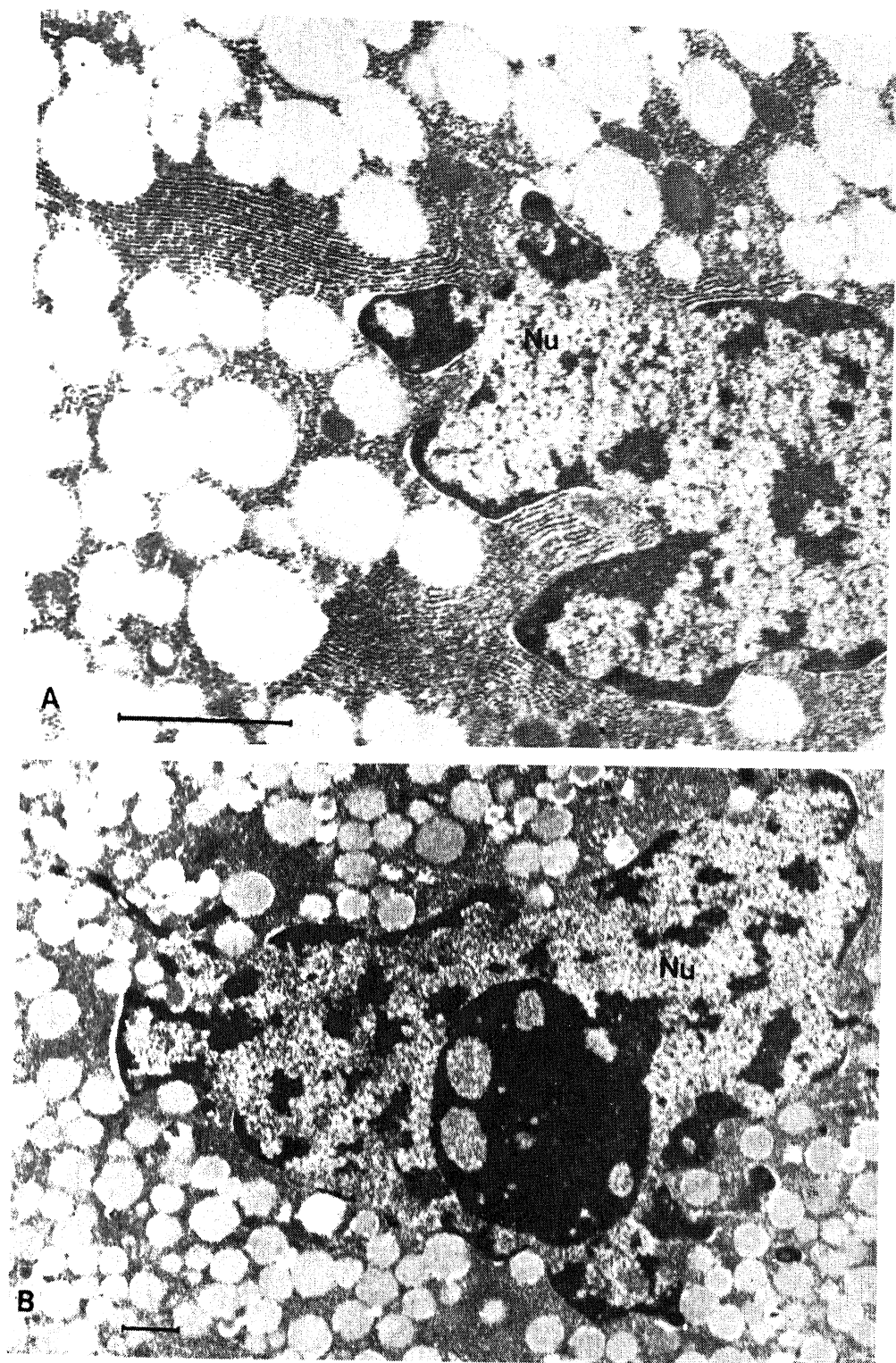
Figure 3. Changes in the nuclear and nucleolar profile with evidence of pinching off of nucleolar material.
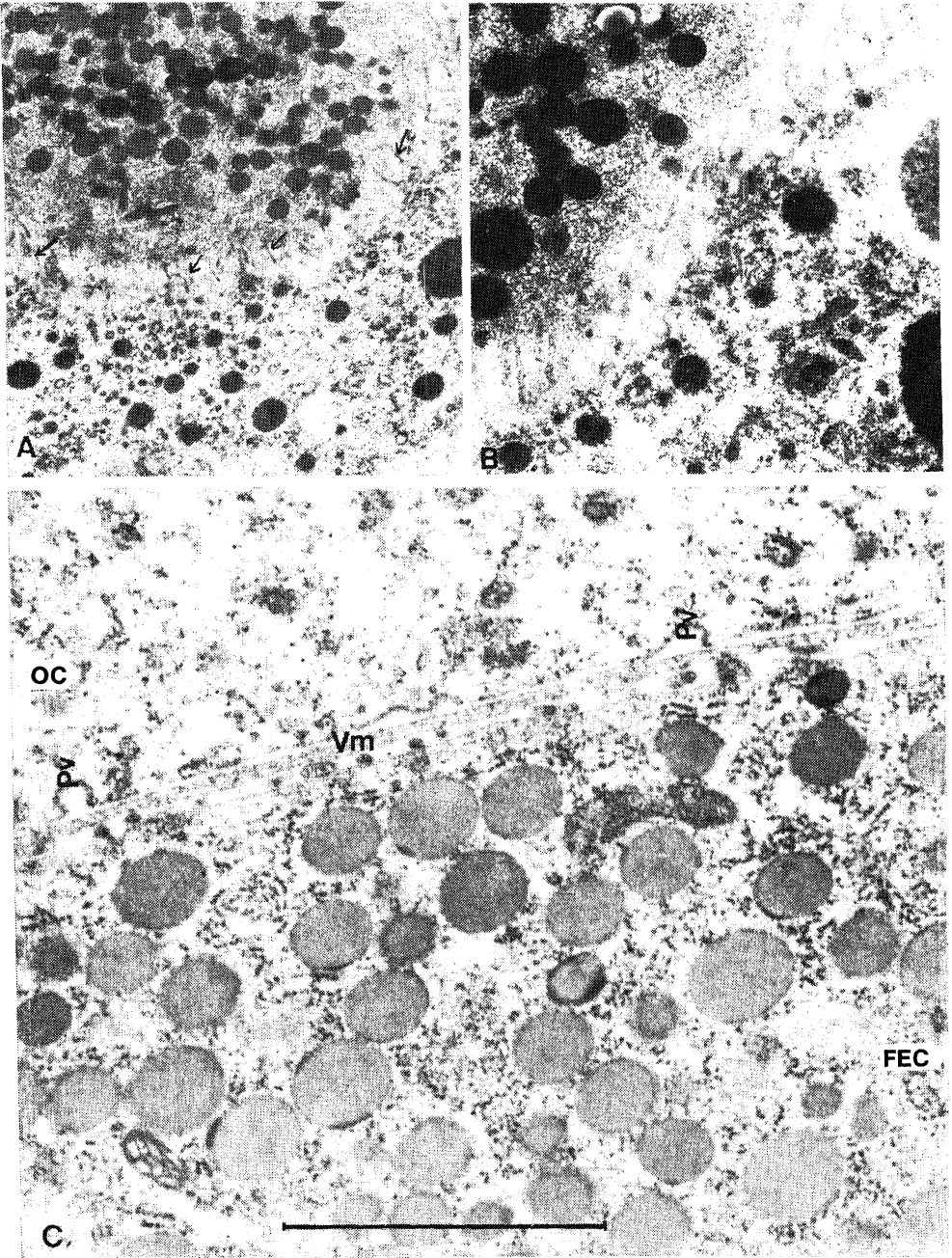
Figure 4. A. Development of tubules (arrows) as well as yolk spheres. **B.** Enlarged view of the tubules and yolk spheres. **C.** Enlarged portion of the follicle cell-oocyte interface showing the formation of vitelline membrane (Vm) and pinocytic vesicles (Pv).

the production of yolk spheres involves vesicle generation, the transformation of vesicle contents into granular material which gradually condenses and finally the elaboration of an enclosing membrane around the developing yolk spheres. The
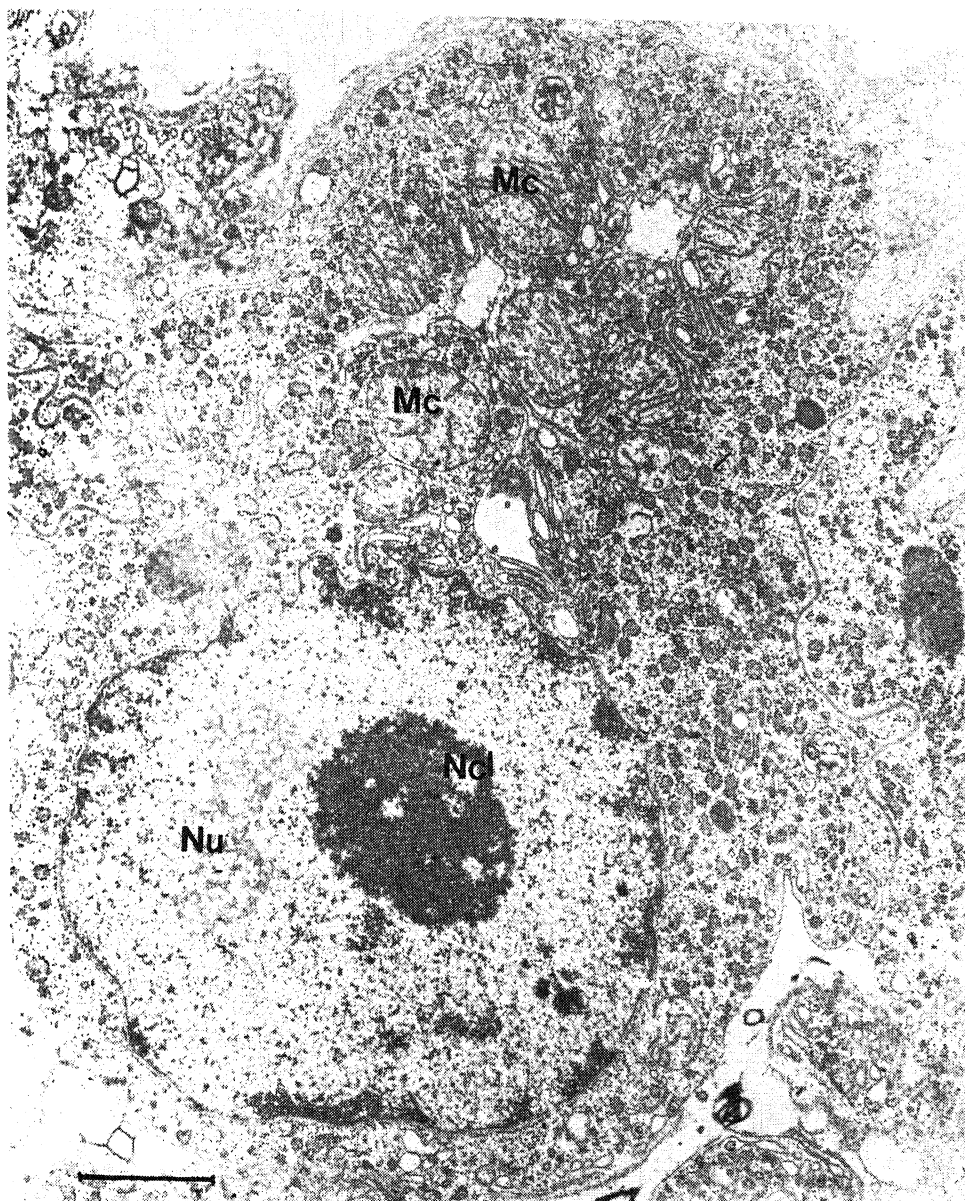
Figure 5. Differentiation of the organelles in the region of the FEC and a portion of the OC.

efficient functioning of the oocyte cortex as a pinocytic system is due to the increased availability of the oocyte surface for the uptake of extracellular material.

4. Discussion

The mode, function and fate of the nucleolar extrusions have been much discussed by Brachet (1957) and Caspersson (1962) who opined that the nucleolus is the site of
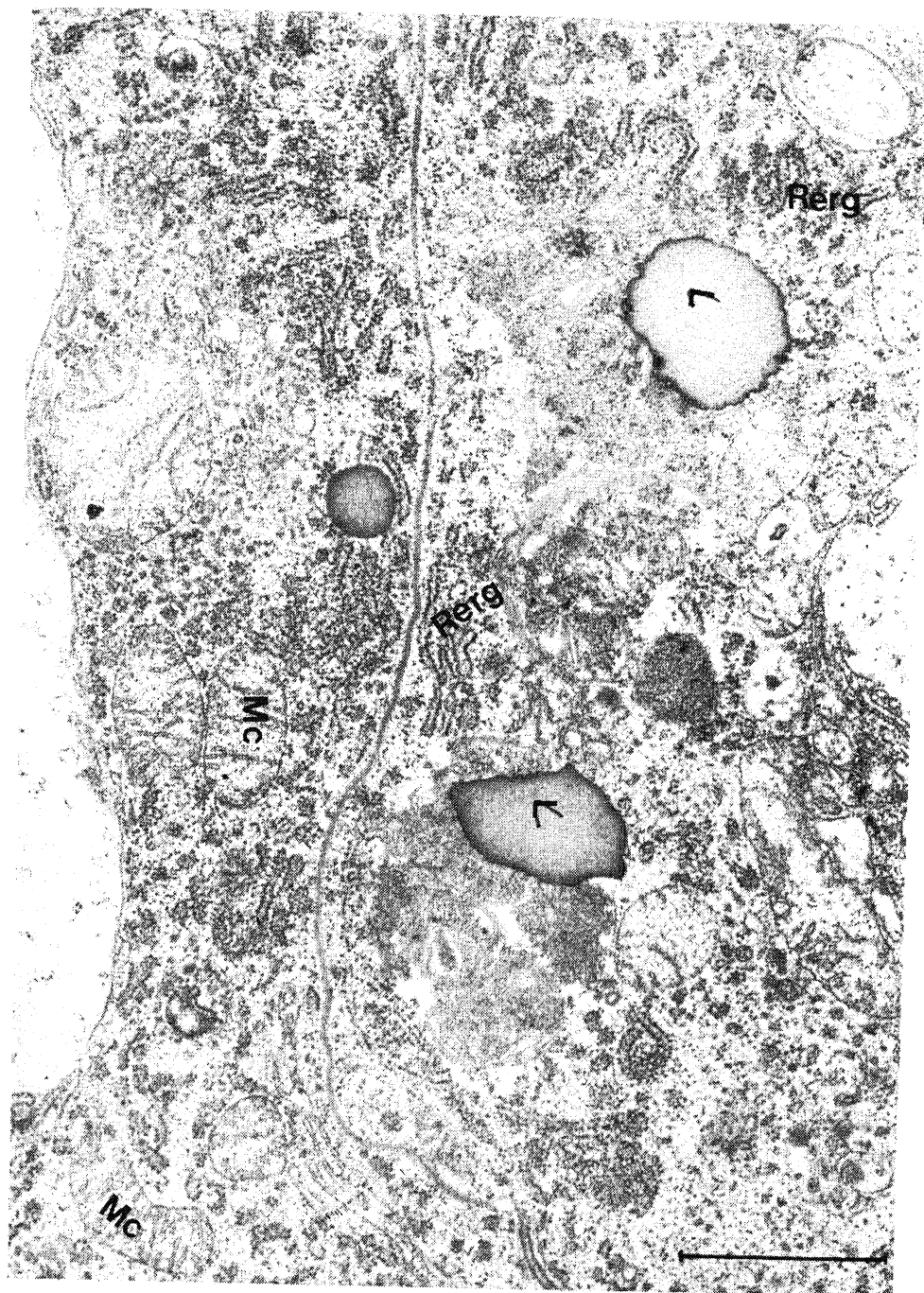
Figure 6. Further enlarged portion of the OC showing the RER lipid droplets, mitochondria, etc.
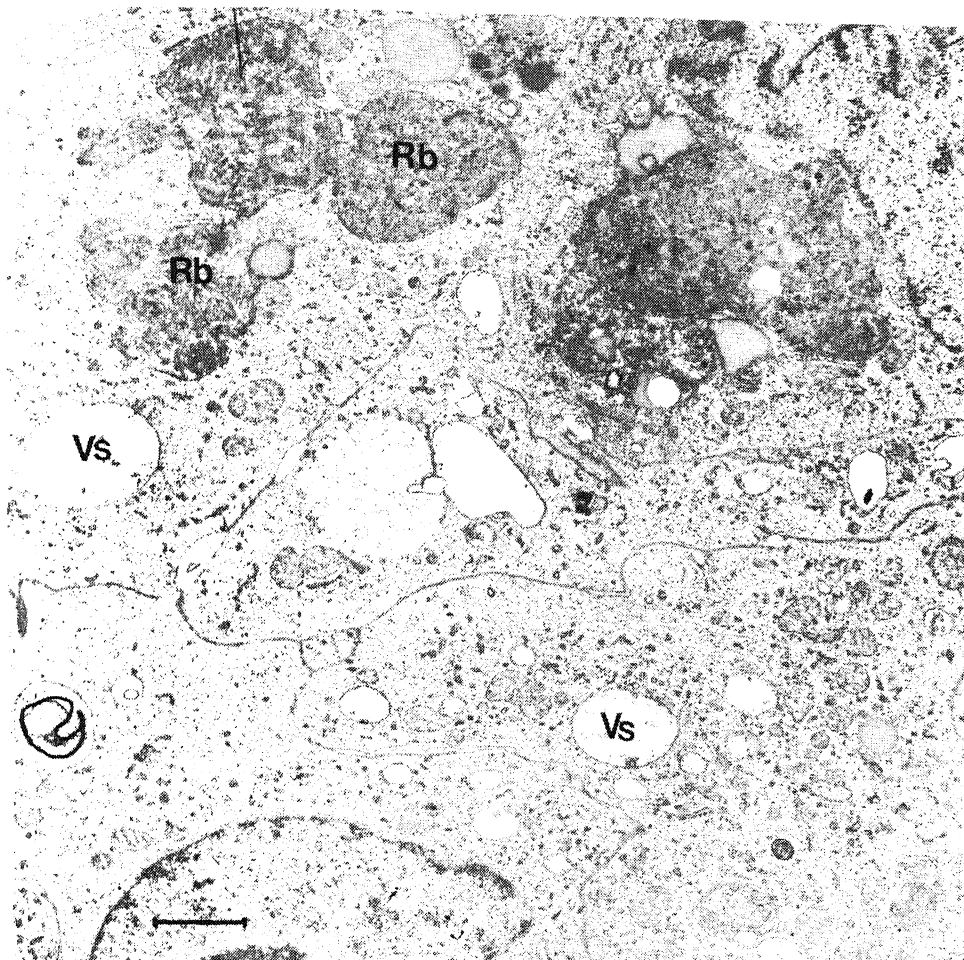
Figure 7. Ultrastructure of the FEC showing a number of refractile bodies. Rb, refractile body; Vs, vesicle.

protein synthesis. The proteins traversing the nuclear envelope becoming located within the cytoplasm in the oocyte of *Periplaneta americana*. Gressen and Threadgold (1962) indicated that the nucleolar emissions move away from the nucleus to the cell periphery, large clear spaces appearing in each extrusion, while Anderson (1964) in his study on oocyte differentiation and vitellogenesis in the roach, *P. americana*, has presented evidence that they are not extruded into the cytoplasm in such large masses. Matzuzaki (1971) in his studies on the panoistic ovarioles of the dobson fly *Protohermes grandis* indicates that 3 or 4 small masses of nucleolar material are dispersed closely opposite to the inner layer of the nuclear membrane. The present observations on *A. ramakrishnae* oocyte provide clear evidence of the occurrence of such nucleolar extrusions and the pinching off of nucleolar material and its subsequent accumulation in the cytoplasm.

It would not be out of place to indicate (Goltzene 1979) that the primary changes taking place during vitellogenesis as visualized in *Locusta migratoria* involve protein

synthesis and storage in RER by transformation of material of nucleolar origin in the oocyte perinuclear cytoplasm as well as by pinocytic activity, accumulation of haemolymph material and secretory activity of the FEC leading to the formation of the vitelline membrane constitute the second stage of activity. Anderson (1964, 1969) has confirmed as a result of studies on dissociation of the oocyte from the follicle epithelium in *P. americana* that the FEC are able to make a protein-like substance that is accumulated in the periphery of the yolk spheres. Since the proteins produced by the follicle cells help in the concentration of haemolymph protein, they are vital for vitellogenesis. The RER and golgi as well as vesicles evident in the FEC have been interpreted as containing secretory products (King and Agarwal 1965). It has been indicated that the OC must maintain a high rate of vesicle formation and fusion throughout the period of yolk formation (Telfer and Smith 1969), an aspect confirmed in studies on the oocyte of *A. ramakrishnae* (Ananthakrishnan 1988a, b). The cisternae of the golgi complex of the FEC appear to elaborate secretory droplets which migrate to the cell surface, become extruded and lie in the space between the oocyte and the FEC. As indicated by Ananthakrishnan (1988a, b) there also takes place a widening of extracellular spaces in the follicular epithelium. Evidence is presented here for the possible incorporation of the follicle cell secretions into the refractile bodies by the oocyte, an aspect supported by Telfer and Smith (1969). The temporal changes in the ultrastructure of the cortical ooplasm have been interpreted as changes possibly due to increased uptake of vitellogenins from the haemolymph (Giorgi and Mazzini 1984). The tubules evident at the pinocytic cortex are believed to be concerned with yolk protein deposition in view of their occasional attachment to yolk spheres. They have also been interpreted as residues of vesicles which have fused and injected their contents into the yolk spheres (Telfer and Smith 1969). The sustained influx of pinocytic products causes stratification of the cortex by the displacement of several cytoplasmic organelles including mitochondria and endoplasmic reticulum. This stratification appears to persist through vitellogenesis, till the periplasm of the mature egg is laid down. Such a stratification is very typical of *A. ramakrishnae* oocyte and follows the appearance in the cortical cytoplasm of the oocyte of small pinocytic vesicles filled with electron dense material immediately below the oocyte. As discussed by Matsuzaki (1971) for panoistic ovaries of dragonfly and cricket, these tiny vesicles or spheres in the peripheral cytoplasm of the oocyte tend to coalesce with each other resulting in larger spheres of protein yolk, which make their way or migrate to the central region of the oocyte.

Well developed, lamellar RER with a large number of ribosomes, many of which are polysomes is characteristic of the follicle cells subsequent to the formation of the vitelline membrane (figure 5). That the follicle cells play an essential role in the formation of the vitelline membrane has been confirmed by several workers and is evidenced by the presence of numerous osmophilic granules accumulating in the space between the oocyte and FEC. In conclusion it may be emphasized that the effective functioning of the OC as a pinocytic system is due to the increased availability of the oocyte surface for the uptake of extracellular material.

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Trophic structure and diversity in some litter inhabiting microarthropods of monoculture and natural forest ecosystems

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Abstract. Temporal changes in species composition of litter insect communities of Shevroys, as well as their trophic diversity involving mycophagous and predatory species are examined with reference to natural forest as well as monoculture systems.

Keywords. Entomoresources; litter ecosystem; monoculture; natural forest; trophic chain.

1. Introduction

The biotic resources of litter ecosystem involving diverse insect species and other microarthropods tend to be highly variable in natural undisturbed and exploited forests as well as in typical monoculture plantations (Ananthakrishnan 1988). The heterogenous forest floor comprising as it does an extension of the structural diversity of its vegetation with a complex of biotic and abiotic components, naturally shows alternations in both quality and quantity of the litter. Intact natural forest ecosystems by the diversity of their flora support more diverse decomposer and consumer systems at various trophic levels than those obtained from man-made monoculture practices which result in truncated decomposer and food chain links resulting in reduced structural complexity. The distribution patterns of the microarthropods in terms of vertical distribution of litter also appear important and inter-specific competition tends to be reduced by vertical distribution as well as horizontal compartmentalization of resources. Except for the detailed taxonomic studies in the litter collembolans by Prabhoo (1971), very little critical work has been done relating to trophic structure in natural forest litter ecosystem in contrast to monoculture litter ecosystems. An attempt has been made to analyse the structural composition and trophic diversity of the entomofauna associated with the litter habitat.

2. Materials and methods

Litter samples from both monoculture and natural forest litter habitats of the Shevroy hills (1,600 m) (Yercaud, Salem District) were collected periodically for a one year period starting from March 1987 at different localities (sites). A known area of one square meter was marked and the population counts of larger insects viz., blattids, earwigs, lygaeids, reduviids, carabids, scarabaeids, etc. were made on the spot and for the population counts of smaller insects viz. collembolans, acarines and anthocorids, the litter samples were collected in polythene bags (42 × 30 cm), tied with rubber bands and then carefully brought to the laboratory. The samples were then run in a series of berlese funnels for a period of 24 h. The floating extracts collected in conical flasks having 70% alcohol were collected with a fine camel hair

brush and then analysed both qualitatively and quantitatively. On the spot assessment of the tree/plant species contributing to the litter was also made.

Utilization of leaf litter for the extraction of entomofauna essentially comprised of *Callitris rhomboidea* (Cupressaceae), *Syzygium cumuni* (Myrtaceae), *Linociera ramiflorea* (Oleaceae), *Vaccinium neilgherrence* (Vacciniaceae), *Jacaranda mimosaefolia* (Bignoniaceae), *Symplocos cochinchinensis* (Symplocaceae), *Terminalia chebula* (Combretaceae), *Grevillea grandulosa* (Proteaceae), etc., from natural undisturbed forests, while monoculture litter involved leaves of eucalyptus, coffee and tea plantations. The temperature and humidity in the different litter zones were measured using digital Digi-sense Thermocouple Thermometer (Cole-Parmer Instrument Co., USA) and Psychrometer probe, respectively.

In order to identify the surface mycoflora of the thrips inhabited sites, the specific feeding sites in the host material were cut into bits, transferred into a 100 ml Erlenmeyer flask containing sterile distilled water (25 ml) over an ultraviolet lamp. This was shaken vigorously so as to bring the surface mycoflora into the sterile distilled water and the process was repeated by transferring the material to 6–7 flasks. This served as the host washings, which were placed on potato dextrose agar/oat meal agar/Czapek's Dox agar media for laboratory culture of the fungi associated with litter. Guts of thrips were dissected out, washed with sterile distilled water and the contents released after slitting open the gut under aseptic conditions, diluted and placed on the same culture medium.

3. Results

3.1 *Entomofauna of monoculture and natural forest litter habitats*

The different components of the litter vary from one type to another, being correlated with the nature of the forests (natural/undisturbed) as well as the vertical distribution from mean sea level (MSL) to different elevations in the Shevroy hills. The entomofauna of natural forest litter habitat as examined (figure 1) not only indicates the total species composition, but also variations at different altitudes and different sites examined. Species. of Collembola, Thysanoptera, Dictyoptera, Psocoptera, Dermaptera and Coleoptera were dominant and clear variations both in terms of species diversity as well as species abundance were evident when such litter insect species were compared from litter at MSL and at altitudes. Collembolans comprising the dominant group ranged from 250–3100/m^2 at MSL and 1800–3700/m^2 at an altitude of 1,600 m, while acarines were the next in order ranging from 55–124/m^2 at MSL and 110–255/m^2 at altitudes. Except for the blattids and reduviids all the other groups like scarabaeids, carabids, psocids, staphylinids, earwigs, anthocorids, lygaeids and thrips showed a dominant trend at altitudes as compared to the natural vegetation stands at MSL.

A comparative assessment of the entomofauna in 3 different monoculture litter habitats viz., eucalyptus, tea and coffee showed considerable variation both in terms of species diversity and species density. Eucalyptus presented a more complex structure as compared to tea and coffee litter, with acarine population always characteristically dominant in eucalyptus litter ranging from 91–180/m^2. Tea litter was totally devoid of such insect groups as scarabaeids and thysanopterans, with a
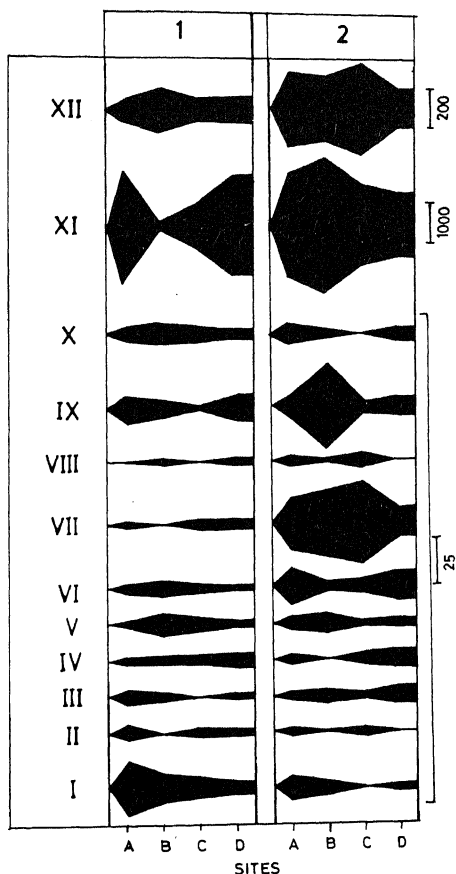
Figure 1. Entomofaunal resources of natural forest litter at MSL (1) and altitude (2).
(I), Blattids; (II), Reduviids; (III), Scarabaeids; (IV), Carabids; (V), Staphylinids; (VI),
Dermapterans; (VII), Psocids; (VIII), Anthocorids; (IX), Lygaeids; (X), Thrips; (XI),
Collembolans; (XII), Mites.
The scale represents number of insects.

very low population of collembolans and acarines. The staphylinids which were not
represented in eucalyptus litter were well represented in the tea litter. The coffee litter
is devoid of such groups as carabids, earwigs and thysanopterans and showed a
very low population of acarines (figure 2).

3.2 *Vertical stratification of litter*

Based on the quality of leaf litter, colonizing insect species vary at the 3 different
layers of the litter habitat. Analysis of the vertical components of the litter elements
shows the top most layer, comprising the predatory food chain, with such insect
groups as Orthoptera, Hemiptera and Coleoptera being represented more. The
intermediate litter layer, where partially decomposed litter is available, the colonizing
insects mainly belonged to the order Thysanoptera, Dermaptera, Coleoptera and
Collembola. The third or transition (humus) layer mainly comprised of the
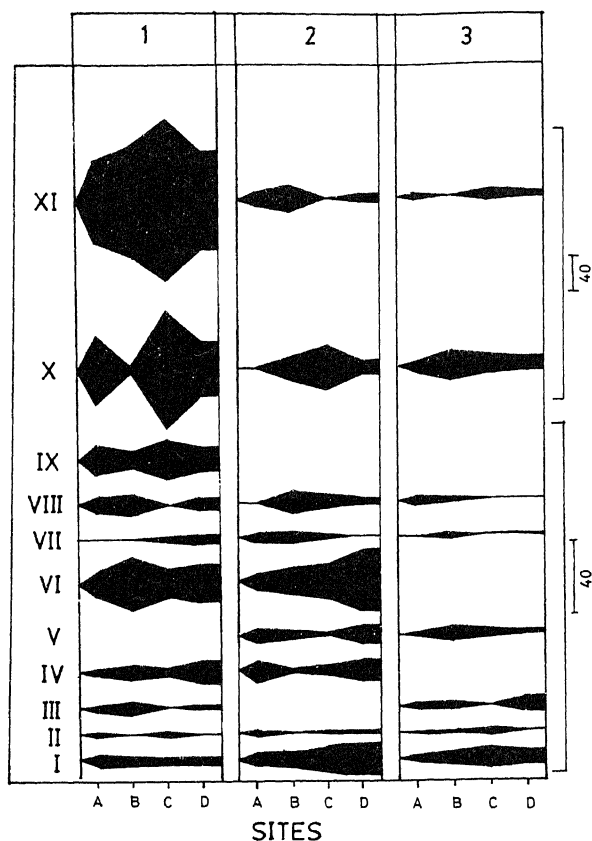
Figure 2. Entomofaunal resources of (1) eucalyptus, (2) tea and (3) coffee litter.
(I) to (VI) as in figure 1; (VII), Anthocorids; (VIII), Lygaeids; (IX), Thrips; (X), Collembolans; (XI), Mites.
The scale represents number of insects.

collembolans and acarines. In the case of the natural forest litter the collembolans tend to dominate all the other groups, while acarines dominated in monoculture litter.

The individual species composition of collembolans was studied in the 3 different litter zones (fresh leaf litter, middle and humus zone) as well as 4 different sampling sites as they form a major component of the entomofauna studied. A total of 8 species were collected and their occurrence varied in the different litter zones. In the top layer species like *Callyntura ceylonica*, *Salina tricolor* (Handschin) and *S. quatturofasciata* (Handschin) were recorded (ranging from 3–65; 4–81 and 0–43/m² respectively). The middle zone is rather complex with a comparatively higher population of such species as *Xenylla* sp., *Hypogastrura* sp., *Cryptophygus thermophylus* and *Tullbergia* sp. dominating all the other collembolans in terms of the population structure. The humus zone is the most important layer which comprised all the species represented in the middle zone, but *Xenylla* sp. appeared to dominate the other collembolans, reaching as high as 2078/m² followed by *Hypogastrura* sp. (410/m²), *C. thermophylus* (320/m²), *Tullbergia* sp. (168/m²) and

Folsomides sp. (96/m²). One characteristic feature of this collembolan population structure is the increase in the population of *Xenylla* sp. always showing a decline in the population of *Hypogastrura* sp. and vice versa. A considerable difference in the temperature at the different zonations was also evident as indicated in figure 3. A higher temperature ranging from 21–23°C was recorded in the surface litter while 20–21° and 19–21°C were recorded in the middle and humus zones respectively.

3.3 *Trophic diversity of litter insects*

The species composition of the natural forest litter in contrast to the monoculture indicates the existence of such potential trophic links involving mycetophagous elements like *Neurothrips indicus* Ananthakrishnan feeding on *Aspergillus niger* and *Penicillium* sp. (Hyphomycetes); *Stigmothrips limpidus* Ananthakrishnan feeding on *Aspergillus niger, Penicillium* sp. (Hyphomycetes) and *Pestalotia* sp. (Coelomycetes); *Ecacanthothrips tibialis* (Ashmead) feeding on *Aspergillus niger* (Hyphomycetes) and *Fusarium oxysporum* (Hyphomycetes), and *Hoplandrothrips flavipes* Bagnall feeding on *Aspergillus* sp. (Hyphomycetes). The sporophagous elements include *Dinothrips sumatrensis* Bagnall feeding on *Verticicladium theobromae* (Coelomycetes); *Elaphrothrips denticollis* (Bagnall) feeding on *Pithomyces* sp., *Aspergillus* sp. (Hyphomycetes) and *Pestalotia* sp. (Coelomycetes); and *Nesothrips* sp. feeding on *Aspergillus* sp. (Hyphomycetes). In the case of eucalyptus litter, individuals of *Priesneriana kabandha* (Ramk.) were noticed feeding on *Cystospora* sp. and *Rhytidhysterium rufula* (Coelomycetes). Apart from collembolans and acarines, such lygaeids as *Metochus uniguttatus* Bergroth, *Graptostethus servus* Fabr. and *Naphiellus dilutus* Thun. served
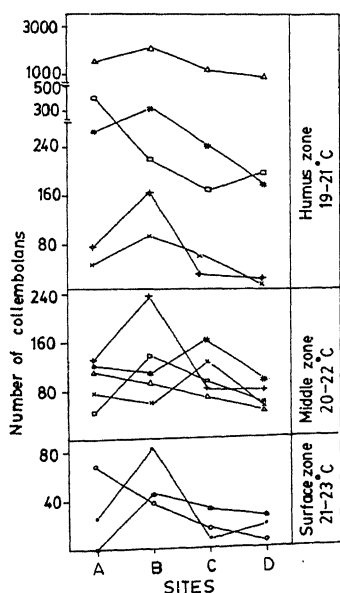
Figure 3. Distribution of collembolan species in the natural forest litter at altitude. (△), *Xenylla* sp.; (□), *Hypogastrura* sp.; (#), *Cryptophygus thermophilus*; (-+-), *Tullbergia* sp.; (×), *Folsomides* sp.; (○), *Callyntrura ceylonica*; (·), *Salina tricolor*; (☉), *S. quatturofasciata*.

as secondary consumers. The earwig species *Foraminolabis sisera* (Burr.), *Eubore*
sp. and *Eparchus insignis* (Haan) and *Labidura riparia* (Pallos) and the reduviids
Pirates affinis Serville, *Acanthaspis coprolagus* Annandale, *Lazarda annulosa* St
Rhinocoris sp. etc., feed on the other arthropods like ants, carabid beetles, termi
and gryllids thereby serving as the top carnivores comprising the predatory fo
chain. The predatory chain is further complicated due to the existence of su
anthocorids as *Cardiastethus* sp., *Scoloposcelis parallelus* (Mots.) and *Or*
maxidentex Ghouri.

In the case of monoculture litter, the trophic chain gets disrupted by the to
absence of several important species at all the trophic levels. The examples incl
the absence of such thysanopterans as *Hóplandrothrips flavipes*, *Elaphrothr*
denticollis, *E. procer*, *Dinothrips sumatrensis* and collembolans as *Tullbergia* :
Cryptophygus sp., *Callyntura* sp. and *Salina* sp. at the primary consumer level of
saprophytic or detritivorous chain. The predatory chains lack such dermapterans
Foraminolabis sisera and *Euborella* sp; anthocorids as *Scoloposcelis parallelus* a
Orius maxidentex and reduviids as *Ectomocoris* sp., *Acanthaspis coprolagus* a
Pirates affinis (figure 4).

4. Discussion

A comparative assessment of the litter fauna associated with a natural forest lit
habitat to that of a monoculture habitat indicates a clear variation not only in
population structure but also in the species complexity. The breakdown of orga
material involves various agencies including the fauna, microflora and the envir
mental factors. There is also a considerable variation in the composition of the li
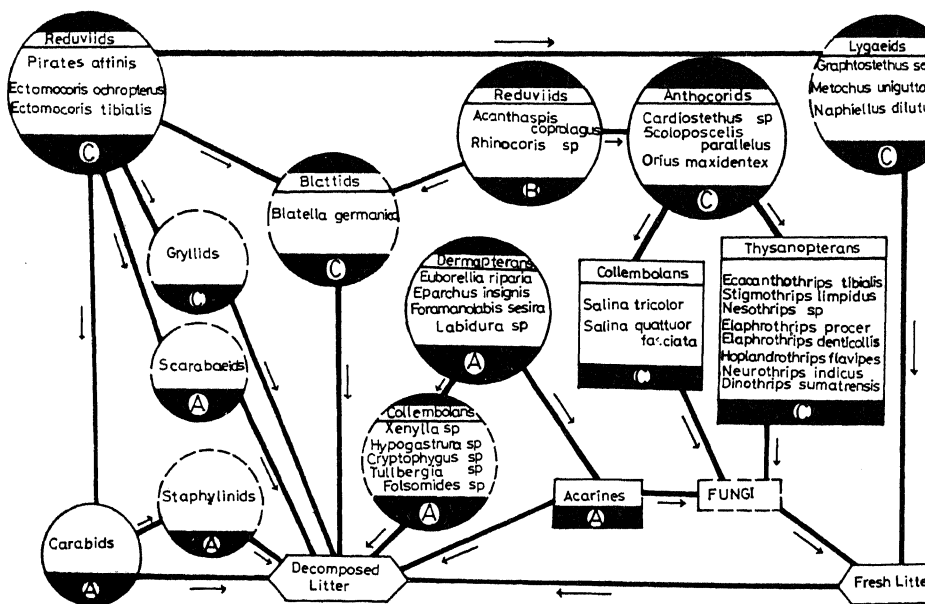
Figure 4. Flow chart indicating the trophic interaction with reference to a typical nat
forest habitat. (A), (B) and (C) represent 3 different litter zones.

horizons overlying the mineral soil, both as a result of variations in the litter fall and because of the differential decomposition of the types of organic matter present. This change in the litter quality in the present study could be attributed mainly to the collembolan and acarine populations which show variation in their abundance. The collembolan population being higher in the natural forest litter adds to the rapid decomposition, while a reduced collembolan population in monoculture results in slow decomposition and breakdown. Lull (1959) compared humus depths in virgin and managed systems and found that the depth of humus involved in managed systems was only slightly less than in corresponding virgin stands. The increased humus occurrence in the virgin stands may be due to the increased population of such microarthropods as acarines and collembolans thereby increasing the decomposition as evident from the present study. Wallwork (1967) also points out that the long term effects of animals with a low population metabolism may be of greater significance than the spasmatic influence of a relatively scarce mesofauna.

The vertical stratification of the types of litter of microorganisms suitable for a particular micro-arthropod species or their immatures may be largely responsible for species distribution pattern in the field. Anderson (1971) while explaining the combination of high species diversity with a comparatively low degree of feeding specificity in detritivore communities, such as Collembola suggested that inter specific competition is possibly reduced not only by different vertical distribution, but also by the horizontal compartmentalization of resources. The vertical stratification of the collembolan species and the other entomofauna in the present study could be due to the vertical stratification of the litter horizons, the nature of the litter, and the microclimatic adaptations of the colonizing species. The abundance of large collembolan forms in the top litter layer and the cryptic forms in the middle and humus zone could also be a behavioural strategy to escape from the predatory arthropods and this differential vertical stratification may be a temporal displacement since many species are not restricted to any one particular layer. Some of the temporal changes in the species composition of populations associated with decomposing litter have been correlated with physical and chemical properties of the litter (Strenzke 1952). Cernova (1971) suggested that certain microarthropods would be used as indicator species for particular stages in the decomposition of manure. The occurrence of higher population of such collembolan species as *Xenylla* sp., *Tullbergia* sp. and *Folsomides* sp. in the present study is a direct indication of a decomposed litter with a thick humus layer. The reduced population of such species on fresh litter may be due to their inability to ingest freshly fallen leaves as indicated by Schaller (1950), where high mortality was encountered, when the collembolans were reared on fresh leaves. However, Dunger (1956) observed feeding of *Folsomia fimeterria* on fresh litter but it was only in traces.

Further the trophic chain in a natural litter habitat is more complex than a monoculture litter and the absence of important trophic links in the monoculture litter indicates the loss of potential bioresources in such monoculture habitats as coffee, tea and eucalyptus.

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Earthworm population dynamics in different jhum fallows developed after slash and burn agriculture in north-eastern India

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Abstract. The population dynamics of earthworms in 3 successional fallows developed after slash and burn agriculture (jhum) at an elevation of 960 m of Meghalaya was analysed. While 0- and 15-yr old fallows had only one species in the soil ecosystem, 5-yr fallow had one additional species apart from those found in the other two fallows. Monthly population fluctuation was related to soil temperature, moisture and litterfall pattern. The rate of mortality in the population was high with a generally low mature population fraction compared with juvenile and immature ones. Different species had different patterns for population fluctuation; this was particularly true in 5-yr old fallow where 3 species co-existed. The significance of these results are discussed.

Keywords. Biomass; earthworm; humid tropics; litter; population; slash and burn agriculture.

1. Introduction

Earthworms play an important role in soil fertility maintenance through their role as an important consumer/decomposer of organic matter (Satchell 1947; Dash 1978; Senapati and Dash 1984). While much information on the role of earthworms in maintenance of temperate soil fertility is available (Edwards and Lofty 1978; Syers and Springett 1984; Lee 1985), the information on tropical/subtropical soils is limited (Edwards and Lofty 1977; Satchell 1983; Watanabe and Ruaysoongnern 1984; Lee 1985), their role in natural ecosystem function in particular is least understood (Dash and Patra 1979; Krishnamoorthy and Vajranabhaiah 1986).

Slash and burn agriculture (jhum) is a major land use of the north-eastern hill region in India (Toky and Ramakrishnan 1981; Mishra and Ramakrishnan 1981). With rapid shortening of the jhum cycle (the intervening fallow phase between two successive croppings on the same site), soil fertility maintenance is a major problem (Ramakrishnan and Toky 1981; Mishra and Ramakrishnan 1983a). Therefore, an understanding of the soil ecosystem function particularly the role of earthworms in maintaining soil fertility is important. The present study, therefore, deals with the population dynamics of earthworms in early successional fallows developed after slash and burn agriculture (jhum) in north-east India.

2. Study area and climate

The study was carried out at Nayabanglow (25° 45″ N and 91° 54″ E) at an altitude of 960 m in the Khasi hills of Meghalaya about 30 km north of Shillong. The pre-cambrian rocks are represented by gneiss, schists and granites. The soil is a red sandy

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loam of laterite origin. The pH ranged from 5–6. The angle of the slopes ranged from 20°–40°.

The climate is typically monsoonic with an average rainfall of 1800 mm of which maximum rainfall occurs between May to September. The average maximum and minimum temperatures during the monsoon were 29° and 17°C, respectively, and during the winter period these were 21° and 4°C respectively (figure 1).

3.   Methods of study

Zero-, 5- and 15-yr old fallows after slash and burn agriculture (3 replicates of each) were identified at Nayabanglow taking care to ensure similar topographic and exposure conditions. The age of the fallows were based on our own observations and also on the basis of records of the village headman. Age was calculated from the time the crop plots were left fallow for natural regeneration.

Soil temperature measurements are an average of 3 random observations at a soil depth of 5 cm and based on 4 monthly observations on each replicate fallow plot. Ten soil samples (0–7 cm depth) randomly collected for chemical analyses from each replicate plot in June 1985 was mixed into a composite one along a transact at regular intervals.

Available phosphorus was measured colorimetrically in the fresh soil sample, by ammonium molybdate method after extraction with Bray and Kurtz's (1945) solution.
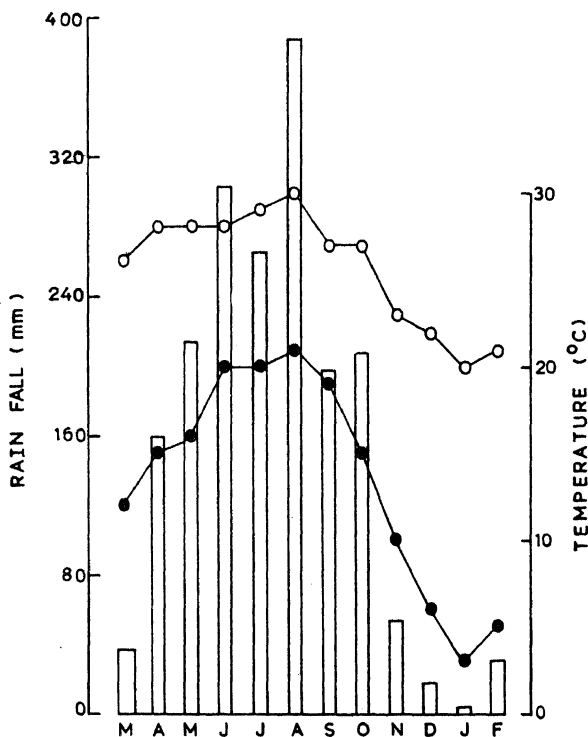
Figure 1. Ombrothermic diagram for the study area. (○), Mean maximum temperature; (●), mean minimum temperature; vertical bars, rainfall.

The soil was then air dried, ground and passed through a 0·2 mm sieve and stored in polythene jars for subsequent analyses. Elemental analyses were carried out following standard procedures (Allen *et al* 1974). Nitrogen was estimated by micro-kjeldahl method, nitrate nitrogen by phenol disulphonic acid method and organic carbon by Walkley-Black method. Calcium and magnesium were determined by EDTA titration method and potassium by the flame emission method after extraction of samples with 1 M ammonium acetate at pH 7.

Density, frequency and cover of the different plant species in the fallows were measured using 1 m² quadrats for herbs and 100 m² quadrats for shrub and tree species. Importance value indices, which is an integrated measure of the relative frequency, relative density and relative basal area of the species was calculated and were based on 20 quadrats at each site, laid along a transact line running down the slope (Kershaw and Looney 1985).

Litter production in different fallows were measured using traps of 1 m² size at 10 random points at each replicate site. Sampling was done at monthly intervals except during the monsoon period when they were made at bi-weekly intervals. The litter samples were dried at 80°C and weighed.

Earthworms were extracted by hand sorting method (Stockli 1928) and kept in 10% formalin. They were subsequently classified based on length into juveniles (<4 cm), immatures non-clitellate (>4<8 cm) and mature (>8 cm). Biomass measurement of the preserved worms was carried out after oven drying at 105°C for 12 h.

4. Results

In 0- and 5-yr old fallows, soil temperature had two distinct peaks one in April-May and another in October followed by a steep fall during January (figure 2). In a 15-yr old fallow, however, temperature fluctuations were less pronounced between March
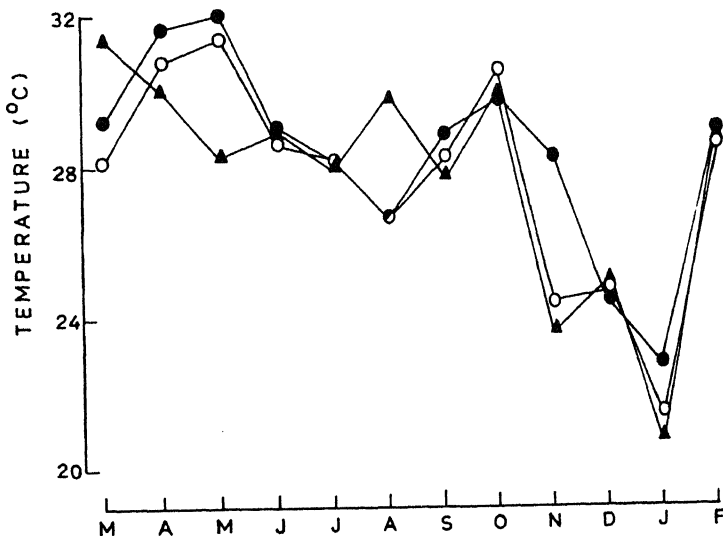
Figure 2. Monthly fluctuations in soil temperatures in different fallows developed after slash and burn agriculture. (●), 0-yr; (○), 5-yr; (▲), 15-yr old fallows.

and October, but dropped sharply during the winter months of November to January.

Soil was acidic, organic carbon and nitrate nitrogen and cations (potassium and calcium) declined with fallow age. On the other hand, total nitrogen, available phosphorus and magnesium improved in 15-yr old fallow (table 1).

In fallows up to 5 years weedy species such as *Mikania micrantha, Arundinella bengalensis, Grewia elastica, Ageratum conyzoides, Panicum maximum, Imperata cylindrica* and *Borreria hispida* formed important components of the community. Besides root and stem sprouts and saplings of shrubs and trees such as that of *Wendlandia tinctoria, Castanea sativa* were present in lesser numbers. In a 15-yr old fallow, besides *Wendlandia tinctoria, Glochidion zeylanicum* and *Garcinia* sp. formed important components of the community (table 2).

Monthly litter production in fallows varied but generally was higher during the dry months of November to March compared to the rainy season. Further litter was generally low in 0-yr old fallow and increased with fallow age (figure 3).

Two different species of earthworms namely *Darwida assamensis* Gates and *Megascolides antrophyes* Stephenson occur in 0- and 15-yr old fallows, respectively. In a 5-yr old fallow, however, both these species occur together along with *Nelloscolex strigosus* Gates.

Monthly fluctuation in the biomass of the total population of earthworms is shown in figure 4. Two major peaks one in May–June and another in September–October were observed. During July–August there was a sharp decline in the biomass. The total population numbers also showed a similar fluctuation as for the biomass and hence not presented here.

The juvenile population fluctuated during different months in all the fallows with corresponding changes in the immature and mature populations (figure 5). Maximum size for the mature population in 0- and 5-yr old fallows occurred in November–December. In both these fallows mature population size fluctuated drastically during different months. On the other hand, the monthly fluctuation in the mature population was less drastic in a 15-yr old fallow and maximum population size was noted in July. Individual species population dynamics in terms of numbers and biomass had a similar pattern and therefore the biomass values are not presented here.

The population structure of *Nelloscolex strigosus* in a 5-yr old fallow had also 3 distinct peaks in April, July and December–January (figure 6). Between these 3 peaks there were phases when the population was absent as during May–June and October–November. In all the species occurring in the different fallows, the activity of earthworms was least during the dry winter months. The population of *Darwida assamensis* showed more drastic fluctuations in both number and biomass in a 5-yr

Table 1. Soil characteristics of different fallows developed after slash and burn agriculture.

Fallow age	pH	Organic carbon (%)	Total N (%)	NO_3^{-N} (mg/100 g)	PO_4^{-P} (mg/100 g)	Cations (m.eq/100 g)		
						K	Ca	mg
0	5·7±0·15	1·83±0·03	0·094±0·001	0·210±0·001	0·338±0·022	0·44±0·03	3·65±0·33	1·54±0·16
5	5·6±0·18	1·68±0·31	0·133±0·013	0·194±0·004	0·342±0·004	0·38±0·02	3·75±0·13	1·64±0·33
15	5·7±0·16	1·46±0·14	0·144±0·013	0·185±0·012	0·354±0·024	0·25±0·02	2·59±0·02	2·04±0·4

Mean values ±SE.

Table 2. Importance value indices (IVI) of plant species in different fallows developed after slash and burn agriculture.

Name of the species	IVI values Fallow age (years)		
	0	5	15
Herbs			
Ageratum conyzoides L.	41·2	27·5	—
Arundinella bengalensis (Spreng) Druce.	64·8	12·2	—
Borreria hispida K schum.	28·9	20·0	—
Grewia elastica Royle.	68·1	5·3	—
Hedychium koronarium Koening.	—	—	22·5
Hedychium sp.	—	1·9	12·9
Imperata cylindrica Beaw.	—	54·4	—
Mikania micrantha H B K	49·0	62·2	—
Panicum maximum Jav.	48·1	15·0	—
Panicum khasianum Munro.	—	12·3	85·2
Thysanolaena maxima (Roxb.) O Ktze	—	14·0	8·0
Shrubs			
Clerodendrum viscosum Vent.	—	2·0	—
Desmodium triflorum D C	—	3·0	—
Eupatorium odoratum Linn.	—	3·0	—
Eurya accuminata D C	—	4·0	6·0
Ficus hispida L.	—	1·0	4·0
Flemengia sp.	—	2·0	2·0
Hedychium spicatum Buch. Ham. ex J E Smith	—	3·0	4·0
Holmskioldia sanguinea Retz.	—	—	6·0
Lantana camera L.	—	4·0	5·4
Leea edgeworthi Sant ap.	—	—	4·0
Lygodium sp.	—	4·0	—
Osbeekia crinata Benth.	—	3·0	2·0
Plectranthus coesta Ham.	—	1·0	2·0
Plectranthus macranthus Hock. F.	—	4·0	—
Rubus ellipticus Sm.	—	—	2·0
Smilex perfoliata Lour.	—	—	4·0
Trees			
Calicarpa arborea Roxb.	—	8·0	5·0
Castanea sativa Miller.	—	12·0	9·0
Emblica officinalis Gaertn.	—	3·0	4·0
Garcina cowa Roxb.	—	3·0	9·0
Glochidion zeylanicum A Juss.	—	—	21·0
Greuria villosa Willd.	—	—	4·0
Olea dentata Wall.	—	—	6·0
Pheobe lanciolata Nees.	—	3·0	—
Quercus dealabata Lindley.	—	9·0	—
Saurauia nepaulensis D C	—	3·0	2·0
Schima wallichii Choisy.	—	8·0	4·0
Wendlandia tinctoria D C	—	15·0	63·0

old fallow than in a 0-yr old fallow. In a 5-yr old fallow, sharp peaking of both immature and mature populations of this species alternated with total absence of the population in the intervening months but with 3 distinct phases for the mature population in May, July and November. In a 0-yr old fallow, however, population of
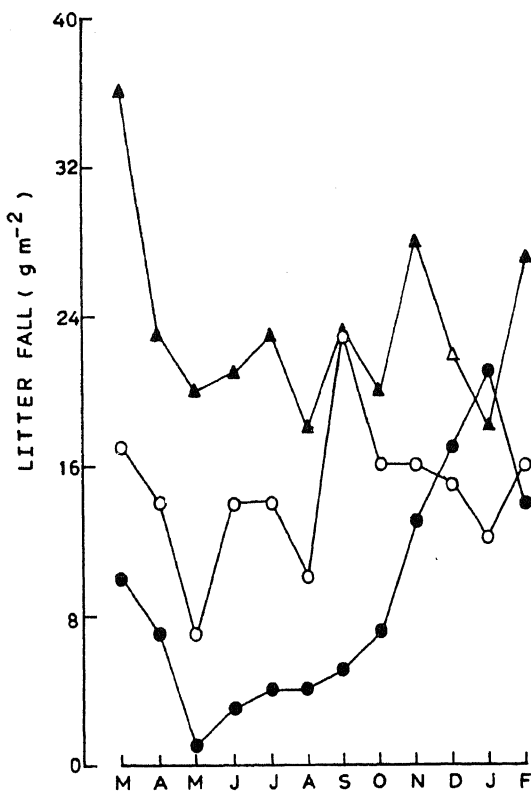
Figure 3. Monthly litterfall pattern in different fallows developed after slash and burn agriculture. (●), 0-yr; (○), 5-yr; (▲), 15-yr old fallows.

this species had two peaks in August for the immature and in December for the mature individuals.

The population of *Megascolides antrophyes* occurring in 5- and 15-yr old fallows showed larger size and flux in the older fallow. In 5-yr old fallow there were distinct periods of absence between May and September. In 15-yr old fallow both mature and immature populations had 3 alternating peaks.

5. Discussion

The fallows under consideration here are those that are developed after slash and burn agriculture (jhum) (Mishra and Ramakrishnan 1981; Toky and Ramakrishnan 1981) and these are therefore, in the processes of recovering after disturbance. During slash and burn agriculture operations, a number of drastic changes occur in the microenvironmental conditions such as availability of light, temperature and humidity on the soil surface (Ramakrishnan *et al* 1981; Ramakrishnan 1985). Further the physicochemical properties of the soil are drastically altered with increased soil pH after the burn, decreased nutrient status of the soil due to volatilization (Ramakrishnan and Toky 1981), and a flush of cations released during the slash and burn (Ramakrishnan and Toky 1981; Mishra and Ramakrishnan 1983b). During the
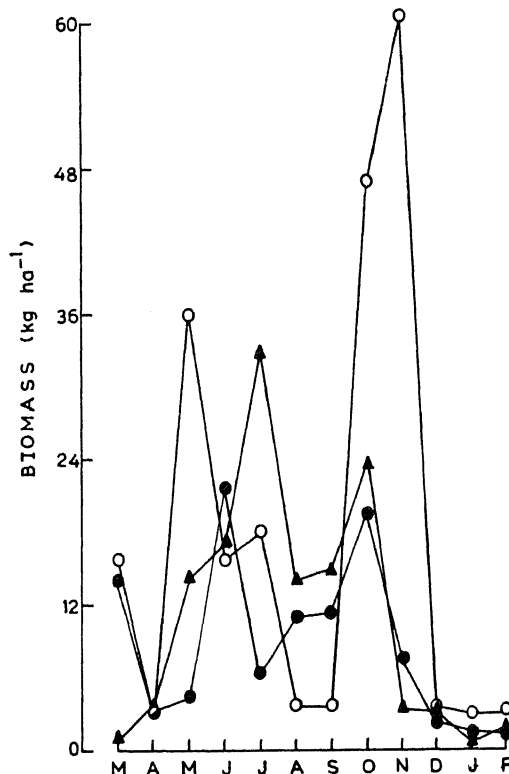
Figure 4. Total monthly biomass of earthworm species in different fallows developed after slash and burn agriculture. (●), 0-yr; (○), 5-yr; (▲), 15-yr old fallows.

cropping phase and the subsequent phases upto 10 years, however, depletion of nutrients occur (Ramakrishnan and Toky 1981; Mishra and Ramakrishnan 1983a). The nutrient recovery occurs only after about 10 years of fallow regrowth when release occurs through litterfall at this time. Therefore, a 15-yr old fallow would be at the recovery phase. The earthworm populations present in 0- to 15-yr old fallows considered here are, therefore, to a certain extent a consequence of such drastic fluctuations in environmental condition.

The generally higher acidity in the fallows is partly related to highly leached soils and slower litter decomposition at an altitude of 960 m. Higher organic carbon in younger fallows may partly be related to application of organic manure during the earlier cropping phase and the rapid release from total death of herbaceous weeds in a 5-yr old fallow, when a shift in community structure occurs from herbs to a shrub and tree dominated phase (Mishra and Ramakrishnan 1983b; Toky and Ramakrishnan 1983). Nitrogen level improves during fallow regrowth because of nitrogen fixation through microbial activity (Saxena and Ramakrishnan 1986). Cations in younger fallows are higher because of release and build-up during slash and burn operation (Ramakrishnan and Toky 1981; Mishra and Ramakrishnan 1983a).

To a certain extent the earthworm population size and structure are related to the humus status of the soil. With little litter production in a 0-yr old fallow, the smaller population size of the only species, namely *Darwida assamensis*, found here is under-
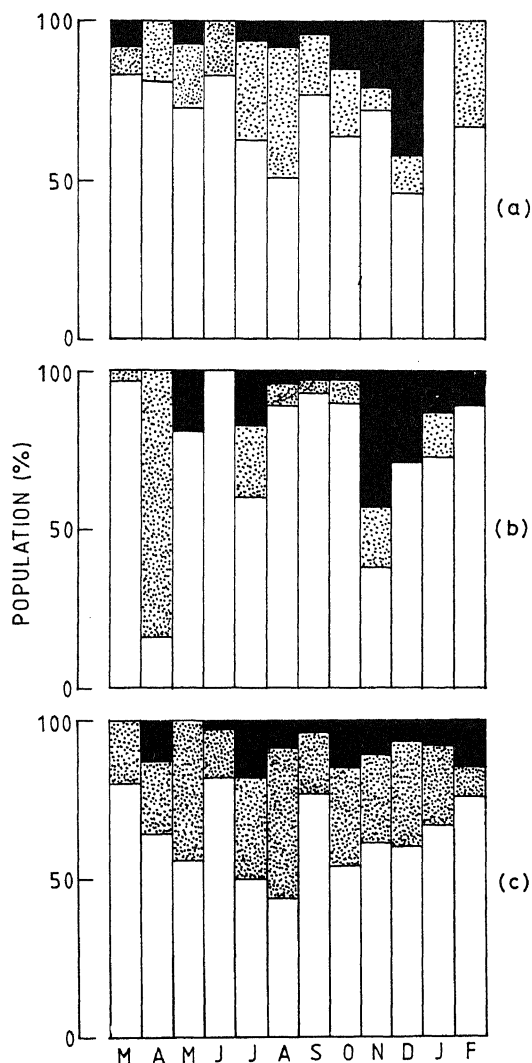
Figure 5. Monthly changes in population structure of earthworms in 0-yr (**a**), 5-yr (**b**) and 15-yr (**c**) old fallows. Open, juvenile; stippled, immature; dark, mature individuals.

standable. With greater release of litter through total death and decay of the herbaceous community in a 5-yr old fallow, the earthworm population size reached its maximum in this fallow. With higher litter production in a 15-yr old fallow, *Megascolides antrophyes* alone present here, had a high population density and biomass.

The monthly pattern in earthworm population followed the litter production pattern and soil temperature fluctuations. With a high litter production during the dry winter and the brief summer period the earthworm population peaked during May–July when soil moisture improved followed by another peaking in October–November as the litter built-up again during the latter part of the monsoon. Very low population levels attained during December–March is partly related to low
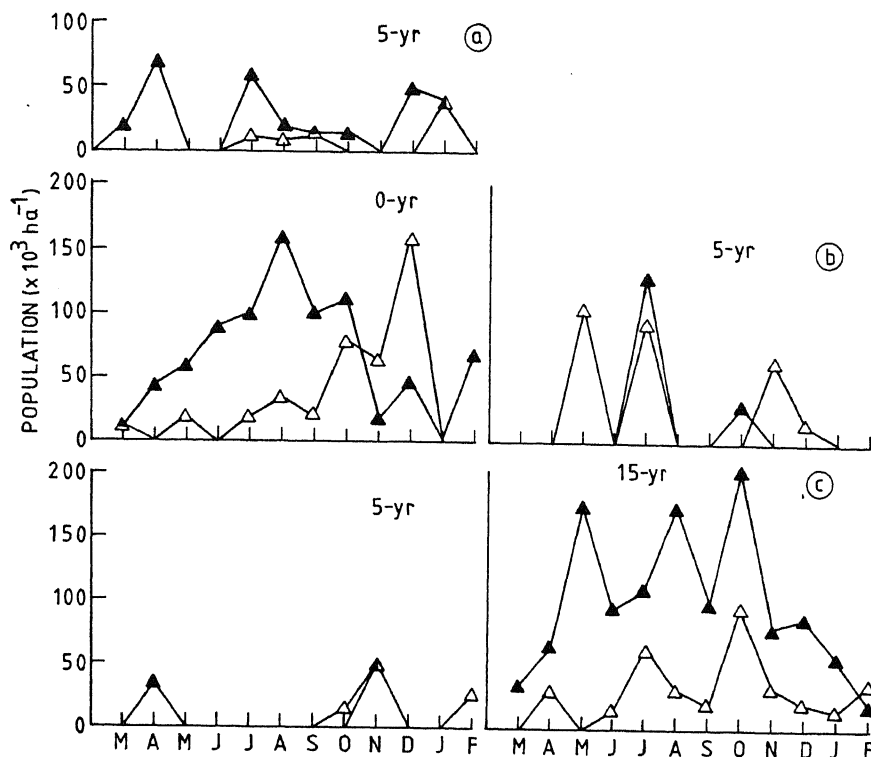
Figure 6. Monthly fluctuations in population size of immature (▲) and mature (△) earthworms. (a) *Nelloscolex strigosus.* (b) *Darwida assamensis.* (c) *Megascolides antrophyes.*

temperature and moisture forcing diapause. That there are a larger proportion of juvenile and immature populations compared to mature ones, during the active breeding period (Evans and Guild 1948) and subsequently suggests that mortality is very high. Obviously the population structure of different species differed as also reported by others (Evans and Guild 1948; Rhee 1967). It is not unlikely that there may be some degree of suppression of one species by another when they occur in a mixture as in a 5-yr old fallow. This, however, needs to be further tested. The values for earthworm population size obtained here (maximum value of 675,000 ha^{-1} in October) is much lower than the values reported for tropical grasslands of Orissa where Dash and Patra (1977) have reported maximum value of up to 800,000 ha^{-1}. Much higher values have been reported from temperate soils (850,000 ha^{-1}, Edwards and Lofty 1977). High rainfall, highly leached thin mountain soil that is acidic and rapid decomposition of litter may be some of the reasons for a low population size recorded here.

Acknowledgement

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Population, biomass and secondary net production of aboveground insects in a temperate grassland

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Abstract. Population, biomass and secondary net production of aboveground insects were studied in a temperate grassland from February 1986 to January 1987. The live shoot biomass varied from $12\cdot4 \pm 0\cdot67$ to $84\cdot8$ g m^{-2} and the aboveground net primary production was $190\cdot3$ g m^{-2} yr^{-1}. A total of 65 species belonging to 33 families of 10 insect orders were collected. Herbivores (77·6%) were the most dominant insects followed by Omnivores (12%), Predators (5%), Saprophages (5%) and Parasites (0·4%). Maximum population density and biomass were $9\cdot8$ m^{-2} and 254 mg m^{-2}, respectively. Cumulative secondary net production was $1\cdot144$ g m^{-2}.

Keywords. Aboveground insects; population and biomass; secondary net production; temperate grassland.

1. Introduction

The grasslands which represent a major terrestrial ecosystem are of great ecological significance particularly because of their role in the production of large herbivores. Detailed studies on population density, biomass and secondary net production have been done both in temperate and tropical grasslands (Smalley 1960; Odum *et al* 1962; Wiegert 1965; Gyllenberg 1969; Nakamura *et al* 1971; Bhatnagar and Pfadt 1973; Gillon 1973, 1976; Lamotte 1975; Vats and Singh 1978; Kaushal and Vats 1984) but data on temperate grasslands in India are lacking. The present study is an attempt to report on population, biomass and secondary net production (SNP) of aboveground insects in a temperate grassland from February 1986 to January 1987.

2. Materials and methods

2.1 *Study area*

The study site is a 5 h piece of grassland situated at Naukuchiatal about 26 km from Nainital at an altitude of 1500 m (29° 20′ N lat. and 70° 35′ E long.). The study site is free from human disturbance except insect herbivory. The study site had an average rainfall of 1711 mm. The mean maximum temperature varied from 12°C (February) to 25·5°C (June) and mean minimum temperature ranged from 8°C (December) to 21·5°C (June) (figure 1). The area is markedly influenced by early monsoon (June) and as per weather conditions, the year is divided into 3 distinct seasons viz. rainy (second fortnight of June to September), winter (November to February) and summer (March to June).

OMBROTHERMIC DIAGRAM FOR NAUKUCHIATAL
(1986-1987)
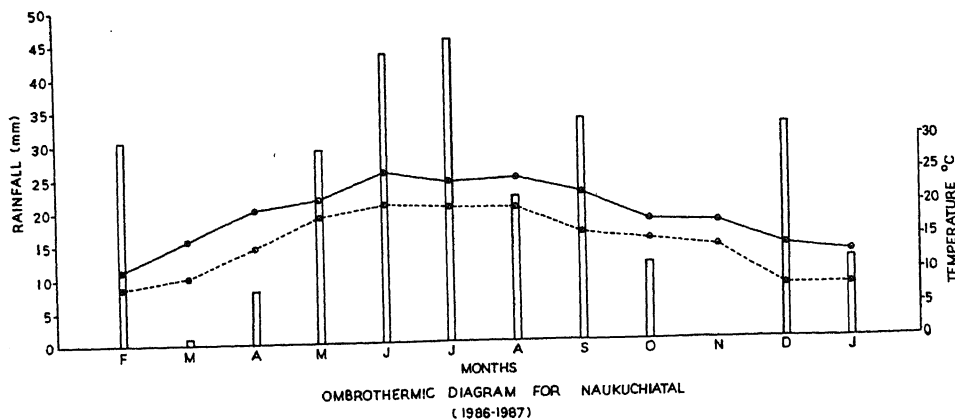
Figure 1. Ombrothermic diagram for Naukuchiatal (1986- 87); ●—●, maximum temperature; ●--●, minimum temperature; □, rainfall.

2.2 *Estimation of population density and biomass*

Population density was studied using removal-trapping method, for this purpose, a cage with an area of 1 m² was constructed with an entrance of 80 × 40 cm on one side 10 cm above ground level. Wire-gauze of 12 meshes per square inch was fixed on all sides except on ground surface. This size of wire-gauze prevented escape of insects from the cage. Random sampling at 15 days interval was done except in winter when it was done monthly, keeping in mind that the cage did not disturb the tips of the vegetation. The trapped insects were killed in jars containing ethyl acetate.

Collected insects were oven-dried to constant weight at 60°C for 72 h. Each specimen was weighed in a single-pan electric balance (0·1 mg accuracy).

Aboveground net primary production was determined by trough analysis on live plus recent dead material (Singh *et al* 1975).

Secondary net production was calculated by using the following formula.

$$P = S + \sum_{i=2}^{n} 1/2(Ni + Ni - 1). \quad (Wi - Wi - 1).$$

Where Ni is the number of herbivores present at time i; Wi is the mean weight per insect at i; i is the sampling time; S is the standing crop at time $i = 1$. It was assumed that $Ni \leqslant Ni - 1$ and $Wi \geqslant Wi - 1$. When Wi was less than $Wi - 1$, the production was considered as zero. This expression was also used by others (Wiegert 1965; Van Hook 1971; Kaushal and Vats 1984).

3. Results and discussion

3.1 *Primary producers*

The grassland is dominated by *Cynodon dactylon, Carax cruciata, Parietaria debilis, Digitaria cruciata, Arundinella neplensis, Symbupogon distance, Trisopogon serrulatus* and *Apluda montica*.The live shoot biomass varied from 12·4 ± 0·67 (February) to 84·8 ± 2·46 g m⁻² (November). The total live shoot biomass in rainy, winter and

summer seasons was 257·8, 217 and 105 g m^{-2}, respectively. Figure 2 shows variations in live and recent dead shoot biomass during the study period.

Above ground net primary production was 190·3 g m^{-2} yr^{-1}. Cumulative net primary production is shown in figure 3.

3.2 *Species composition and trophic components*

A total of 65 species belonging to 33 families of 10 orders were collected (table 1). Maximum number of species (21) belonged to Coleoptera followed by Orthoptera, Heteroptera, Diptera and Hymenoptera while in terms of number of individuals, Heteroptera (259) was the most dominant insect order.
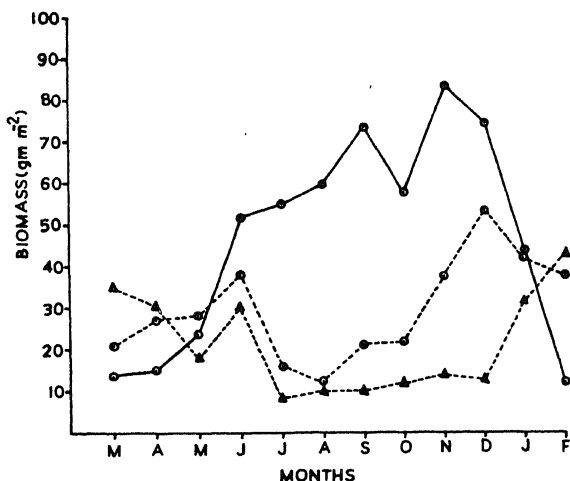
Figure 2. Variation in the live and dead shoot biomass (1986–87); ⊙—⊙, live shoot biomass; ⊙– – – –⊙ dead shoot; ▲– – – –▲, ground dead.
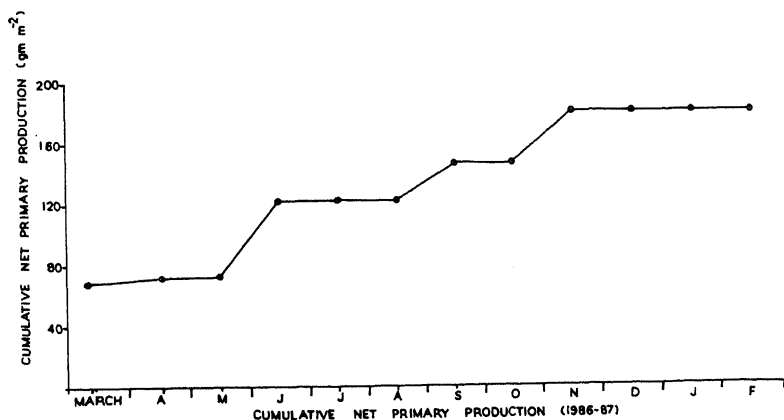
Figure 3. Cumulative net primary production (1986–87).

Table 1. Species composition, trophic components, number of individuals and per cent contribution during 1986–87.

Taxon	Trophic level*	Total No. of individuals	Percentage of total
Orthoptera			
Acrididae			
Phlaeoba ramkrishni Bol	Herbivore	6	1·16
Euprepocnemis roseus Uvarove	,,	6	1·16
Tylotropidius varicornis Walk	,,	13	2·51
Spathosternum calignosum W	,,	36	6·96
Spathosternum strigulatum W	,,	4	0·77
Syrtacanthacris sp.	,,	3	0·58
Unidentified	,,	14	2·71
Tettigonidae			
Perella insignis Bol	,,	18	3·48
Gryllidae			
Gryllus domesticus Linn	Omnivore	7	1·35
Gryllus sp.	,,	8	1·55
Coleoptera			
Scarabaeidae			
Onitis virens Lansb	Saprophage	1	0·19
Serica-aserica sp.	Herbivore	1	0·19
Sisyphus subsidens Walk	Saprophage	2	0·39
Chiloloba acuta Wied	,,	1	0·19
Malendra sp.	,,	1	0·19
Brachmina coriacea Hope	,,	1	0·19
Popillia discalis Walk	Herbivore	5	0·97
Curculionidae			
Myllocerus sp.	Herbivore	2	0·39
Coccinellidae			
Coccinella 7-punctata L	Predator	4	0·77
Chrysomelidae			
Mimastra cynaura Hope	Herbivore	1	0·19
Ceratobasis nair Lacord	,,	1	0·19
Haplosoma sexmaculata Jack	,,	1	0·19
Colosoposoma semicostatum Jack	,,	5	0·97
Lema sp.	,,	2	0·39
Lema lacordairei Baly	,,	1	0·19
Tenebrionidae			
Gonocephalum sp.	,,	1	0·19
Gonocephalum simulatrix Fairm	,,	2	0·39
Carabidae			
Acinopus indicola Bates	Predator	1	0·19
Distichus planus Bonella	,,	2	0·39
Cantharidae			
Mylabris variabilis Poll	Herbivore	1	0·19
Unidentified	Saprophage	2	0·39
Heteroptera			
Lygaeidae			
Graptostethus argentatus F	Herbivore	69	13·35
Dygaeus equestris Linn.	,,	1	0·19

Table 1.   (*Contd.*)

Taxon	Trophic level	Total No. of individuals	Percentage of total
Pentatomidae			
Carbula insocia Walk	,,	162	31·33
Cydnus sp.	,,	1	0·19
Reduviidae			
Coronus spiniscutis Reut	Predator	1	0·19
Coreidae			
Dalader acuticosta Army and Serve	Herbivore	2	0·39
Pyrrhocoridae			
Dysdercus cingulatus LHG	,,	2	0·39
Capsidae			
Megacoelum sp	,,	1	0·19
Unidentified	,,	20	3·87
Homoptera			
Jassidae			
Botrogonia albicans	,,	2	0·39
Cercopidae			
Callitettix vessicolor	,,	1	0·19
Hymenoptera			
Chalcididae			
Brachymeria sp.	Parasite	2	0·39
Vespidae			
Polistes chinensis Fabr	Predator	2	0·39
Polistes maculipennis Sauss	,,	3	0·58
Vespa auraria Smith	,,	1	0·19
Apidae			
Apis indica	Herbivore	4	0·77
Apis sp.	,,	1	0·19
Bombus sp.	,,	4	0·77
Formicidae			
Odontopnera transversa Smith	Omnivore	22	4·26
Diptera			
Asilidae			
Philodicus femoralis Ric	Predator	6	1·16
Bombyliidae			
Argyra distigena Vid	Saprophage	1	0·19
Syrphidae			
Chrysotoxum baphyrus Walk	Herbivore	1	0·19
Syrphus serarius Wied	,,	1	0·19
Calliphoridae			
Lucilia sericata	Saprophage	2	0·39
Lucilia sp.	,	1	0·19
Tachinidae			
Exorista sp.	,,	11	2·13
Trycolyga sorbillans D	,,	1	0·19
Lepidoptera			
Noctuidae			
Chalciope hyppasia Cram	Herbivore	3	0·58

Table 1. *Contd.*

Taxon	Trophic level	Total No. of individuals	Percentage of total
Saturniidae			
Erebia hyagriva M	,,	1	0·19
Pieridae			
Eurema hecabe	,,	2	0·39
Blattoidea			
Blattidae			
Mareta sp.	Omnivore	11	2·13
Unidentified	,,	14	2·71
Odonata			
Libellulidae			
Trithimus annulata Beeson	Predator	6	1·16
Dermoptera			
Forficulidae			
Anisolabis annulipes Luc	Saprophage	2	0·39
Total		517	100·00

*Little (1963), Lefroy (1984).

On the basis of number of individuals collected, herbivores were the most dominant (77·6%) followed by Omnivores (12%), predators (5%), saprophages (5%) and parasites (0·4%).

Evans and Murdoch (1968) reported 1584 species in an abandoned farmland in south-eastern Michigan; 117 species in the sown grassland (Igarshi 1973); 514 species in alfalfa community (Pimental and Wheeler 1973); 126 species in a grassland at Kurukshetra (Kaushal and Vats 1984). The number of species decreases with an increase in elevation (Janzen 1973).

Physical structure of the grasses affects the number of species (Gillan 1983). Insect orders followed different patterns on the basis of their dominance in different habitats (Gillon and Gillon 1974; Janzen and Pond 1975; Kaushal and Vats 1984). Vegetation cover influences the number of species in a grassland at Pawnee site (Bhatnagar and Pfadt 1973).

Herbivores are the most dominant individuals in all types of marshes and grasslands as in the present study also (Davis and Gray 1966; Evans and Murdoch 1968; Van Hook 1971; Kaushal and Vats 1984) except saprophages which were dominant in a Savanna, Ivory Coast (Gillon and Gillon 1974).

3.3 *Population density and biomass*

Data on population density and biomass are plotted in figure. 4. Population density varied from $0·7 \text{ m}^{-2}$ (January) to $9·8 \text{ m}^{-2}$ (May). Maximum population density was obtained in the middle of summer season because of favourable conditions of moisture and optimum atmospheric temperature (20–25°C). Minimum values were obtained in the dry period (winters) when temperature was low, scanty rainfall and low productivity.
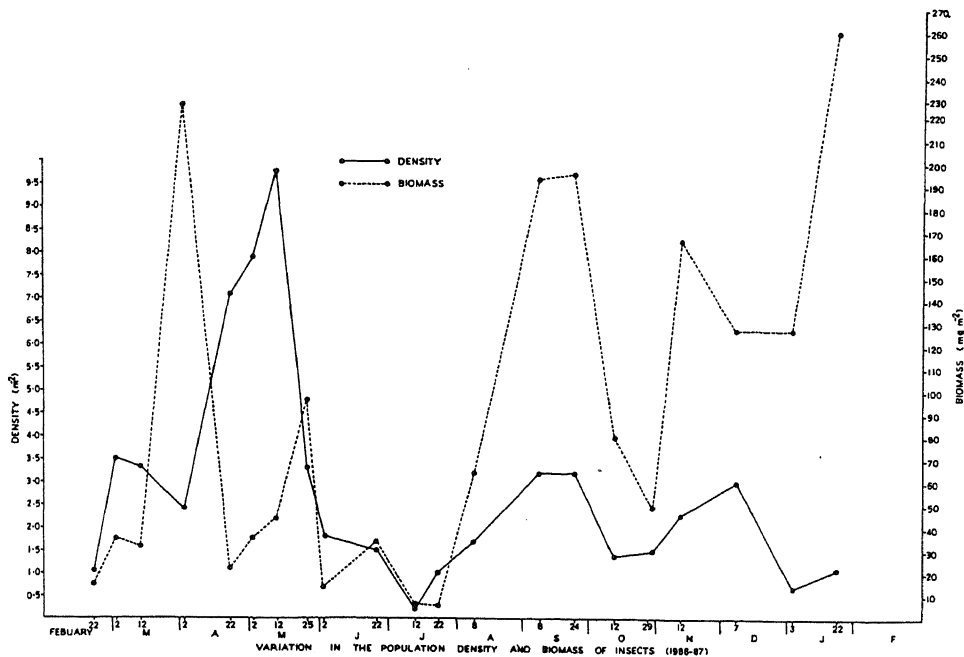
Figure 4. Variation in the population density and biomass of insects (1986–87).

There are relatively a few studies on the population density of insects. Maximum density in a Savannah at Ivory Coast was $31 \cdot 19 \, m^{-2}$ (Gillon and Gillon 1974); $19 \cdot 32 \, m^{-2}$ in Borassus palm Savannah, Lampto (Lamotte 1975); $40 \cdot 5 \, m^{-2}$ in a grassland at Kurukshetra (Kaushal and Vats 1984). In comparison, maximum value $(9 \cdot 8 \, m^{-2})$ in the present study is quite low.

Decrease in population in the dry season may be due to quality of food (Blend and Swayze 1973) and also climatic conditions (Edwards 1960; Pradhan and Peswani 1961; Putnam 1962; Wiegert 1964). Davis and Gray (1966), Dempster (1975) and Wolda (1978) reported low density of insects in the dry seasons.

The biomass values varied from $5 \cdot 7 \, mg \, m^{-2}$ (July) to $254 \, mg \, m^{-2}$ (January). Maximum values of biomass were obtained towards the beginning of winter season when more adults with higher weight were collected. Maximum biomass values in a Savannah at Ivory Coast was $485 \cdot 74 \, mg \, m^{-2}$ (Gillon and Gillon 1974); $687 \cdot 07 \, mg \, m^{-2}$ at Lampto (Lamotte 1975); $5880 \, mg \, m^{-2}$ in a grassland (Kaushal and Vats 1984). In comparison, maximum biomass $(254 \cdot 0 \, mg \, m^{-2})$ recorded in the present study is quite low because of low population density.

Thus it can be concluded that the number of species, population density and biomass are influenced by species composition of the vegetation, quality and quantity of food and abiotic factors.

3.4 SNP

Time series data on population density and biomass of herbivores are used in the estimation of SNP (figure 5). Cumulative SNP in the present study was $1 \cdot 144 \, g \, m^{-2}$.
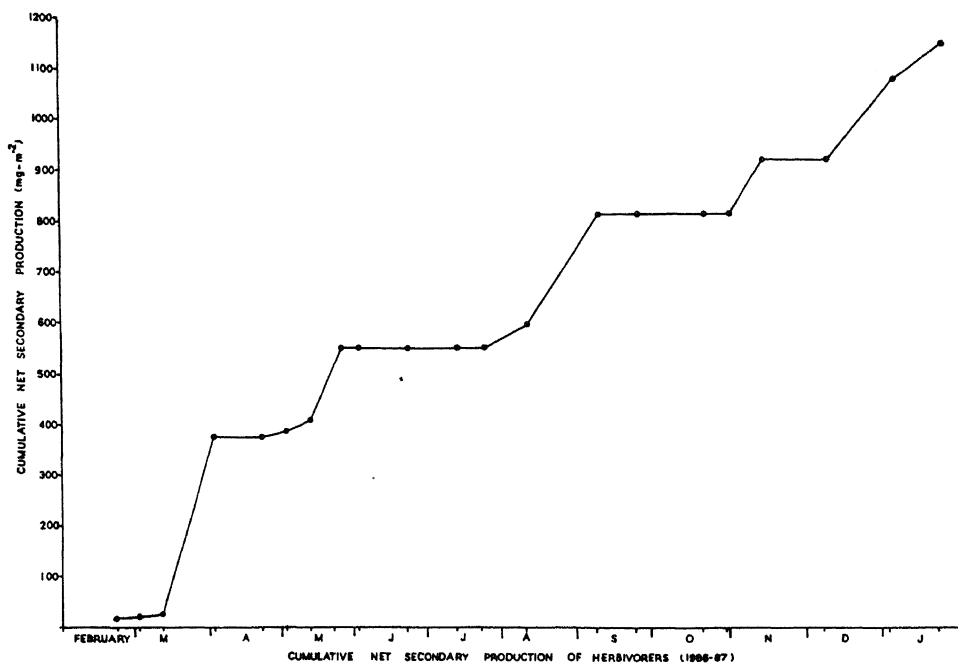
Figure 5. Cumulative net secondary production of herbivores (1986–87).

In comparison, SNP of grasshoppers in the oldfield ecosystem at Michigan was only 122 mg m^{-2} in 1959, 71·7 mg m^{-2} in 1960 and for alfalfa ecosystem, it was 878·9 mg m^{-2} (Wiegert 1965). Riegert and Varley (1973) reported 45·97, 24·5 and 47·29 mg m^{-2} SNP in the unstressed, burned and grazed plots, respectively. SNP was 393·75 mg m^{-2} in 1969 and 80 mg m^{-2} in 1971 in the natural grassland (Riegert *et al* 1974). Kaushal and Vats (1984) reported 4·19 g m^{-2} and 2·31 g m^{-2} SNP on stands I and II, respectively in a tropical grassland. SNP thus varies with population density and biomass in different habitats.

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The subcellular morphology of avian adrenal medulla

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Abstract. The electron microscopic study of the avian adrenal medulla have shown that in most birds, the epinephrine and norepinephrine cells can be differentiated based on the morphology, size, opacity and the fine structures of their granules. However, the functional characterization of some medullary cells, other than epinephrine and norepinephrine remains unresolved to this date. Among the avian adrenals studied, it is only in the domestic fowl that the separate cellular origin of epinephrine and norepinephrine cells have been established.

Earlier studies on the manner of release of secretory granules from the adrenal catecholamine storing cells in birds have led one to infer that exocytosis is only mode of discharge of granules. Surprisingly this is not apparent in some of the reports obtained in recent years. Hence the release mechanism of the adrenomedullary hormones need to be further explored ultrastructurally.

Keywords. Epinephrine; norepinephrine; chromaffin; transmission electron microscopy; scanning electron microscopy; birds.

1. Introduction

The avian adrenal gland is unique, in the sense, that it reveals a complete intermingling of interrenal and medullary tissues (Vesterguard and Willeberg 1978). This avian attribute, is in sharp contrast to the organised adrenal structure of all other homoiothermic vertebrates, where the central medullary portion is encapsulated by adrenal cortex, which itself contains at least 3 concentric strata.

Significant contributions by Chester-Jones (1957), DeRoos (1963), Frankel (1970), Wells and Wight (1971), Assenmacher (1973), Hodges (1974), Sturkie (1976) and Holmes and Cronshaw (1980) have vastly increased the existing knowledge of the avian adrenal gland. Further, the research activities of Ghosh and his collaborators (see Ghosh 1977, 1980) have made important contributions to the study of avian adrenal medulla using biochemical, histological and histochemical techniques in control and experimentally manipulated animals. Unfortunately, the avian adrenal medulla, unlike the avian interrenal tissue, has been little studied ultrastructurally. As a consequence, no comprehensive review of the cellular morphology of the avian medullary cells at the ultrastructural level is available. It is felt that such a pursuit will be rewarding, as characteristic information obtained from the birds, will amplify ideas regarding adrenomedullary endocrinology of homoiothermic vertebrates.

Hence, the objective of this review is essentially to present the current status of knowledge of the avian adrenomedullary ultrastructure. To our knowledge this is the first attempt of its kind, devoted to this vital aspect of avian endocrinology.

In birds and mammals the adrenals are a pair of oval, pear shaped or triangular glands yellow or orange in colour. The glands are located bilaterally at the anterior pole of the metanephric kidneys, just anterior to the post-caval vein. The avian

adrenal gland like that of most higher vertebrates, is formed of two components—the cortex (interrenal) and medulla (chromaffin tissue), which are of different origins and exhibit distinct functions (Assenmacher 1973). As in other vertebrates the avian medulla also secrete catecholamines, epinephrine (E) and norepinephrine (NE).

Ghosh and his collaborators (see Ghosh 1977, 1980), studied the relative proportions of E and NE secreting cells and cellular areas in the adrenal medulla of a number of birds representing several orders and families. This data accrued from cytochemical and spectrophotometric methods reveal an extreme range of relative concentrations of E and NE cells, hitherto unreported in homoiothermic and poikilothermic vertebrates. This profound variation of medullary E/NE ratio in birds, suggest a distinct evolutionary pattern (Ghosh 1979).

2.   Ultrastructural studies

In birds, light microscopic studies so far have not helped in identifying the adrenomedullary cell boundaries. However, the nuclear orientation pattern of medullary patches elucidate the somewhat irregular arrangement of cells. The medullary cell clusters are always associated with blood spaces. The more basophilic adrenomedullary cells have fine cytoplasmic granules. Maitra and Ghosh (1980), have differentiated two tinctorially different cell types in the adrenal medulla of *Psittacula cyanocephala* by application of Wood's (1963) modified technique. Densely granulated reddish brown E secretory cell-islets are observed to be accompanied by clusters of NE containing cells, which are homogenously yellowish in colour, relatively smaller in size and fewer in number. Unmyelinated nerves penetrate the adrenal medulla (Carmichael *et al* 1985) and one synaptic terminal may innervate 3 E or NE cells (Unsicker 1973). Two cellular types have been differentiated in the domestic fowl electron microscopically, based on the morphology of granules (Kano 1959; Fujita and Machino 1962; Chungsamarnyart and Fujioka 1982) and Gentoo Penguin (Cuello 1970). The NE cells contain granules of very irregular size and shape, while the E ones are regular spherical and small (Cuello 1970). In the red-vented bulbuls (figure 1) the E cells have larger granules and are not uniformly oval or round, like that of NE cells (Carmichael *et al* 1987). Guzsal and Hassan (1975) identified 4 types of chromaffin cells, based on the morphology of their granules. They reported the presence of spherical E type, spherical and ellipsoidal NE type, heterogenous type of dark cells and also the transitory sympathicoblasts.

During the early seventies, the chromaffin cells of the adrenal gland of almost all vertebrates were classified, based on the electron densities of the chromaffin granules.

Figures 1–5.   **1.** Electron micrographs of adrenal chromaffin cells from red-vented bulbul. The dense vesicles in the left contain NE and the larger, less dense vesicles contain E ($\times$ 10,530).   **2.** TEM of adrenal medulla of rose-ringed parakeet, showing denser NE containing vesicles (curved arrow) and the slightly larger E containing vesicles (arrow) [calibration bar = 1 μm].   **3.** Micrograph from snipe sampled in late February. Fewer electron-dense cores are seen in chromaffin vesicles. The cytoplasm contains several empty vacuoles ($\times$ 25,000).   **4.** The surfacial study of adrenal of kingfisher showing leafy coral like aggregates (calibration—see marker in accompanying photograph).   **5.** SEM of parakeet chromaffin cell, showing conglomeration of bleb like granules in the blood space. The endothelium has rufflings and breakages at points from which the blebs are discharged (calibration bar = 2 μm).
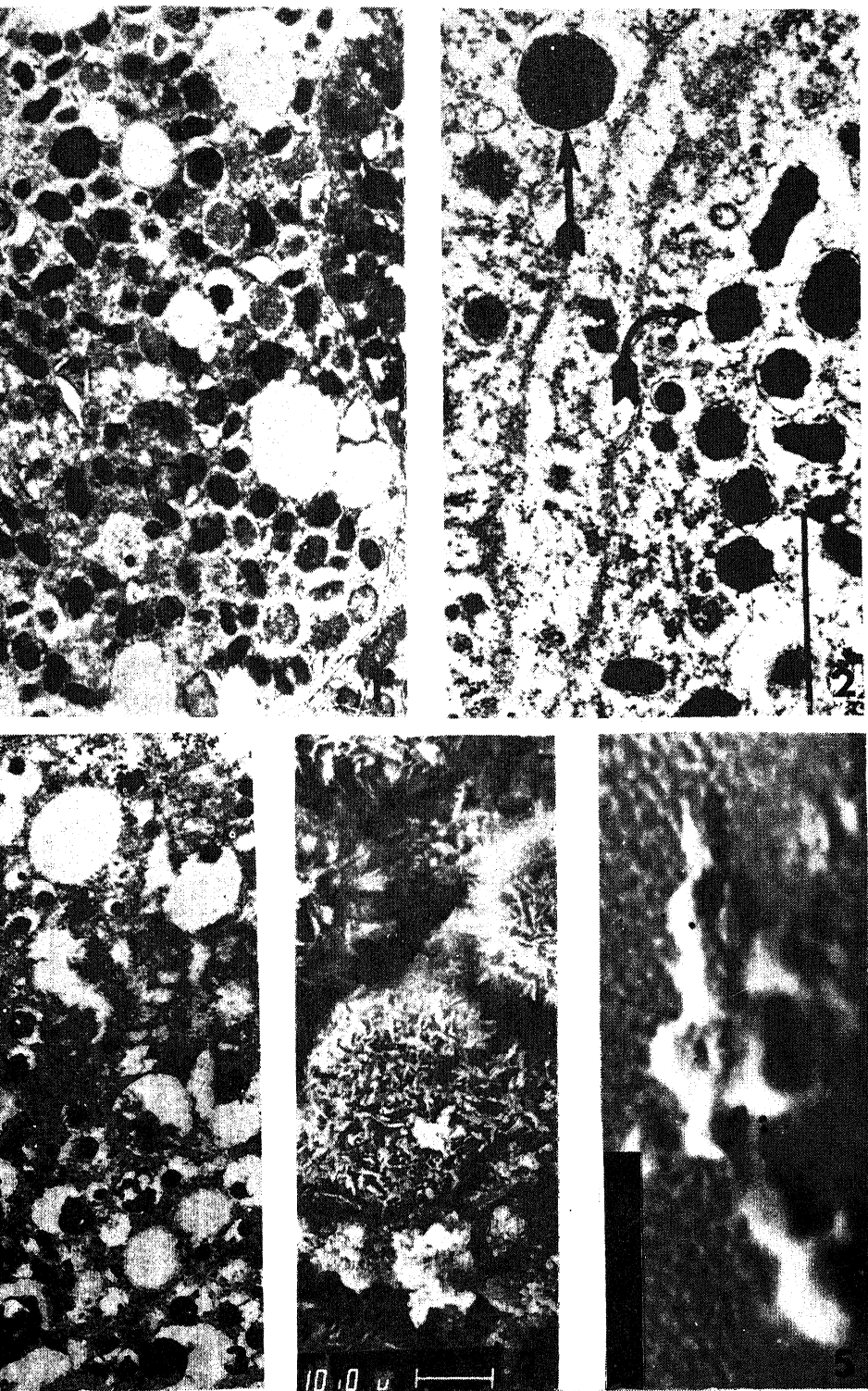
Figures 1–5.

They were described as NE cells, containing dense granule and E cells, containing less dense granules. Earlier studies have revealed that the NE cells are preponderant in the adrenal medulla of fowl (Kano 1959), while the opposite has been observed in the Gentoo Penguin (Cuello 1970). Recently Carmichael *et al* (1987) also observed that in bulbuls there were more E cells than in pigeons.

Unsicker (1973) made a comprehensive study of the adrenal glands of 15 bird species. He noted that the E and NE cells could be distinguished from each other by the structure of their electron microscopically revealed granules. In all the species, E cells prevailed. The E cell granules of Corvidae and some other Passerines, resemble the pituitary pars intermedia, because of their incomplete membranes and the low electron density of their contents.

In Pekin drakes (*Anas platyrhynchus*), Cronshaw *et al* (1974) observed large population of electron opaque neurosecretory granules in the adrenal medulla, and could not differentiate the E and NE cells. On the other hand Guzsal and Hassan (1975) confirmed the separate existence of E and NE cells in the ducks and geese.

Research activities of Carmichael *et al* (1983) have led to the differentiation of the chromaffin cells of rose ringed parakeets (*Psittacula krameri*), based on the density of their secretory vesicles—the dense vesicular NE and less denser E types (figure 2). All the vesicles are surrounded by a limiting membrane. In *Pycnonotus cafer* and *Columba livia* (see Carmichael *et al* 1987), transmission electron microscopic (TEM) studies revealed two types of cells each containing membrane bound vesicles. The E vesicles were less dense than NE ones.

Interestingly enough the effect of migration on the chromaffin cells have only been reported from the snipe (*Capella gallinago*) among the migratory birds by Carmichael *et al* (1985). They observed that specimens collected in fall, had more dense cytoplasm and numerically greater chromaffin vesicles per cell, with low catecholamine contents. They also reported that in late February, there was a remarkable variation, in the size of the chromaffin vesicles with the cytoplasm containing several empty vacuoles (figure 3).

In mammals it is well established that ultrastructurally determined granular sizes vary in different species. In cat, rat and man, the E are larger than the NE (Coupland 1971). However, Yates *et al* (1962) isolated in the Syrian hamster, the peripheral NE cells, containing mainly large granules (average 200 nm), and central E cells containing small granules (average 100 nm).

During the last two decades the size variations of the chromaffin granules have also been utilized as an important criteria for observing the E and NE cells in birds as in mammals. The average size of the NE granules in domestic fowl is considerably greater (224 nm) than that of E (168 nm) (Coupland 1971). Further, it has been reported that NE storing granules show spherical bodies arranged in chains or shells with a diameter of about 20–27 nm. E granules on the other hand show a diffuse fine internal granularity which may appear as reticulum. Both E and NE granules have electron dense central or eccentric osmiophilic deposit, which appear as mass of numerous fine granules, surrounded by a light halo and a limiting membrane. The catecholamine storing granules lie close to the Golgi apparatus and the outer membranes of the granules originate from the same.

In ducks and geese Guzsal and Hassan (1975) have reported E granules of 50–200 nm diameter, with different degrees of electron densities. The NE vesicles are 150–500 nm in diameter, and contain various amounts of diversely arranged dense

substance. The so called 'dark cells' have tightly arranged, large, highly electron dense cytoplasmic granules. The fourth chromaffin cell type, the sympathicoblasts are equipped with small processes.

In the rose-ringed parakeet (figure 2), the chromaffin granules are similar to that of domestic fowl (Carmichael *et al* 1983), while in migratory snipe (figure 3) only NE type chromaffin vesicles have been observed (Carmichael *et al* 1985). The recent TEM studies of pigeons and bulbuls reveal two distinct cell types. In pigeons the granules of the E and NE cells do not differ much sizewise, whereas in bulbuls the E granules are larger (figure 1).

3. Cytoplasmic organelles of E and NE cells

The TEM studies have elucidated some interesting interspecific variations in the cytoarchitectures of the chromaffin cells. However, very little noticeable differences have been observed between the E and NE cell organelles of the same species.

In ducks and geese (Guzsal and Hassan 1975) the mitochondria are oval and have lamellated cristae in the E and NE cells. The Golgi are sacculated in both the cells. The ribosomal material is however more in the E cells. On the other hand, tubular cristae are found in the mitochondria of E and NE cells of the domestic fowl (Chungsamarnyart and Fujioka 1982) with uniformly dispersed ribosomes. The Golgi complex consists of cisternae and vesicular components. Dense bodies occur near the Golgi area.

The E cells of Passerine birds have mitochondria with specifically arranged cristae. Large, cytosomes and inclusions of lipo-fuchsin pigments are found in the gulls (*Larus ribibundus* and *L. argentatus*).

4. Release mechanism of chromaffin granules

In mammals it is already well established that the chromaffin granules secrete their contents by exocytosis. During this process the granules, first fuse with the plasma membrane and subsequently escape (Diner 1967; Søvik 1972). In birds, the release mechanism of the catecholamine secreting granules is not well known and represents a major grey area in our knowledge of avian adrenomedullary physiology (Ghosh 1977).

Ghosh and Ghosh (1969), as a result of their investigation on the role of MAO inhibitors in reserpine induced catecholamine secretion in pigeons, suggested that in birds the release mechanism of catecholamine is different from that which is known in mammals.

Unsicker (1973), however, claimed that exocytosis is the only secretory mechanism of adrenomedullary cells in the aves. In contrast to this, Chungsamarnyart and Fujioka (1982) observed exocytosis occurring very rarely in the intact, control and reserpinized domestic fowl.

During reserpine induced degranulation in domestic fowl, Chungsamarnyart and Fujioka (1982) found that chromaffin granular release was preceded by a process of small and large vacuole formation. The granules are subsequently released into the vacuoles. The vacuoles when they come in contact with the cell membranes, release

their contents outside. Small pits suggesting micropinocytosis, are found around the membranes of opened vacuoles.

In rose-ringed parakeet, the electron microscopic studies (Carmichael *et al* 1983) did not reveal ultrastructural evidences of vesicular breakdown. Hence this does not support the earlier report in rats (Clementi and Zocche 1963).

In insulin induced hypoglycemia (Carmichael *et al* 1987) the NE cells of pigeons are frequently found to be depleted of their vesicles. The remaining vesicles further become eccentric with increase in intracytoplasmic vacuolation. The nuclei become crenated with rough endoplasmic reticulum becoming more prominent. In insulin treated bulbuls, there was no biochemical evidence of catecholamine depletion from the gland and the electron dense vesicles are less altered in appearance. The intracytoplasmic vacuoles in the chromaffin cells of both the birds become prominent.

5. Cell specificity of E and NE granules

Earlier electron microscopic explorations have revealed the presence of specific E and NE storing cells in the adrenal medulla of fowls (Coupland 1971, 1972; Unsicker 1973; Chungsamarnyart and Fujioka 1982), ducks and geese (Guzsal and Hassan 1975) and rose-ringed parakeets, pigeons and bulbuls (Carmichael *et al* 1983, 1987) with no evidence of the presence of mixtures of two types of granules within a single chromaffin cell. Ultrastructural investigations of the embryonic chick adrenal medulla, also reveal two cell types representing E and NE containing cells (Hall and Hughes 1970). In contrast to the above, in Pekin drake—*Anas platyrhynchus* (Cronshaw *et al* 1974) and migratory snipe—*Capella gallinago* (Carmichael *et al* 1987) failed to differentiate between E and NE granules in the chromaffin cells, ultrastructurally. Previous cytochemical reports from this laboratory have shown a differential distribution of catecholhormones in individual medullary cords of pigeons and cuckoos; E being found to be localized in the peripheral portions of the cords while NE in the core (Ghosh 1977). Despite this being the general feature in both these species, some cells show positive reactions for both the hormones. Studies on reserpine action on pigeon adrenal medulla also bespeak of a parallel existence of the two hormones in a common cellular area (Ghosh and Ghosh 1963).

6. Embryonic development of E and NE cells

Hall and Hughes (1970) during their study of developing adrenal gland of chicks, noted that medullary cells occur as single cell at 10 days and coalesce in groups by 18 days. Catecholamine containing granules are a prominent feature of medullary cells at all ages. Two cellular types could be distinguished in the medulla after 17 days of incubation. These may represent E and NE cells. During development, the medullary cell membranes become more closely applied to each other and clumps of such cells become more frequent with increasing embryonic age. In birds, as in mammals, very little endoplasmic reticulum is found in either cortex or medulla at any age.

Smitten (1965, 1972) studied ultrastructurally the secretory cycle of adreno-medullary cells during embryogenesis of chick. The period of synthesis and storage of medullary hormones was reported to be characterized by a reconstruction of membraneous system of the cell.

7. Scanning electron microscopy

The recent SEM studies of adrenal medulla of some birds like wood pecker, kingfisher, parakeet and common snipe (Guha B, Pal D, Carmichael S W and Ghosh A, unpublished results), have helped to elucidate interesting 3-dimensional surfacial information of the adrenal parenchyma cells. The SEM study of intact glands revealed a fine membraneous cover on all the gland surfaces, which are characterized by convolutions and deep furrows. Cords fenestrated by vascular channels with conspicuous fibrous elements are also notable. Certain features like blebs, cords, globules, granular particulates, filamentums and coral like plots have been identified (figure 4) on the adrenal surfaces which interestingly enough, vary from species to species.

Semi-thin section study of parakeet adrenal medulla under SEM, reveal medullary cells containing subcellular particles. Prominent cell features are microvilli like projections, which connect the nucleus with the surrounding cytoplasm. The medullary cells appear as islands of granule laden cytoplasm with single central or eccentric nucleus. Smooth surfaced, round, oval and often cylindrical granular vesicles are distinctive features of chromaffin cells. The vesicles appear to fuse with one another so that irregular bodies are noticed.

The chromaffin granules are voided off from the cells as bleb like particles into the blood spaces. Several blebs later converge and coalesce to form beaded conglomerates (figure 5). The otherwise thick but smooth endothelium show ruffling and breakages at points from which the blebs are discharged.

8. Concluding remarks

It must be admitted that compared to the tremendous advances in recent decades on mammalian adrenal medulla, the volume of information accrued in birds is definitely meagre.

The review on the TEM level studies of avian adrenal medulla, does reveal that in birds, the E and NE cells can be differentiated, based on the morphology, size, opacity and fine structure of their granules. However, since the aforesaid information are gathered from investigations of a restricted number of species, it may be rather premature to make too many generalizations based on them.

It is also worth noting that the results of TEM studies in birds, in contrast to that in the mammals, have failed to resolve, whether E and NE cells originate from the same or two separate cell types of the adrenal medulla.

Of interest, is the newer differentiation of the chromaffin cells in mammals that have resulted from TEM studies. Gorgas and Bock (1976) described 4 chromaffin cell types in mice. Unsicker *et al* (1978) classified the SGC cells of guinea pigs into NE positive and NE negative. Intermediate types have been reported to exist between SGC cells and NE positive and negative, identified as intermediate E and intermediate NE, according to the contents of light and dark granules. In birds, Unsicker (1973) reported from several species, small granules containing SGC cells. Guzsal and Hassan (1975) in addition to the E and NE cells, also identified a variant of NE cells, the so called 'dark cells' and the transitory sympathicoblast in geese and ducks. Nevertheless, the functional characterization of some medullary cellular components other than E and NE remain still to be explored fully in aves.

The recent preliminary studies on the adrenal gland of common birds at the SEM level, have resulted in unravelling certain fascinating aspects of adrenal cells. However, proper endocrinological significance of all the features and clear delineation of glandular areas into medulla and cortex could not be made in all species at this stage.

Linked with the earlier findings, it may be pertinent to note that the chromaffin granules in mammals, in addition to catecholamines, are reported to contain certain nucleotides and calcium in high concentrations (Winkler 1976). In depth studies by Carmichael and Smith (1978) have revealed the existence of tubular channels for transport of high energy nucleotides from mitochondria to catecholamine storage vesicles in rats. Since information of the above nature, is decidedly lacking in birds, further researches in these specific areas may be very rewarding.

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Functional response of *Eocanthecona furcellata* (Wolff.) (Heteroptera: Pentatomidae) in relation to prey density and defence with reference to its prey *Latoia lepida* (Cramer) (Lepidoptera: Lemacodidae)

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Abstract. The functional responses of *Eocanthecona furcellata* (Wolff.) that predates on its caterpillar prey, *Latoia lepida* (Cramer), a sporadic pest of many agricultural crop plants are presented using Holling's 'disc' equation. The results indicate the importance of prey density and defence in influencing the attack response of the predator. Further, the phytophagous habits of this predatory bug and its ability to derive nourishment from plant sources during periods of prey absence are also discussed.

Keywords. Predator; functional response; pentatomid.

1. Introduction

The potential of *Eocanthecona furcellata* (Wolff.), a polyphagous insect predator, as a natural control agent of insect pests has been documented (David and Basheer 1961; Ghorpade 1972; Rai 1978). *E. furcellata* is an effective predator of the slug caterpillar, *Latoia lepida* (Cramer), a pest of several crops. However, little is known of its predatory efficiency and functional response with reference to its prey, *L. lepida*. Interestingly, like many predatory heteropteran bugs *E. furcellata* is also known to feed on plants. The results presented here pertain to the functional responses of the adults of *E. furcellata* in relation to prey density and defence of *L. lepida* using Holling (1959) 'disc' equation, besides describing the nature of the phytophagous habit of *E. furcellata*.

2. Materials and Methods

Nymphs and adults of *E. furcellata* were collected from *Cassia* sp. and mango trees from private gardens in Madras, and a colony of *E. furcellata* was raised from these collections in the laboratory. For culturing the predators glass troughs (25 × 10 cm) were used and their open tops were covered with muslin cloth for proper ventilation. Young mango leaves, provided inside the predator rearing troughs, served as oviposition sites. Eggs and young larval stages of *L. lepida* were collected from mango plants and were reared in the laboratory using glass chimneys with mango foliage. Developmental stages of the prey were determined based on the total length and head width.

2.1 *Functional response tests*

The objective of the experiment was to find out (i) the prey consumption rate by the predator at different prey densities with various stages in a restricted *milieu* in the laboratory and (ii) the influence of prey defence on the functional response of the

predator. The prey insect were packed loosely in the jar with mango foliage, that were changed periodically, since the leaves were not preferred when dry. The prey were randomly distributed in the foliage of each jar and the larvae killed by the predator were replaced daily and the prey number was maintained at all densities. Both males and females were taken equally in replicating the tests for each prey density and at each prey stage level.

To evaluate the influence of prey defence on the functional response of the predator, experiments were repeated with 'defenceless' larvae. A small metal pin was used to hold the thoracic segment of each larva so that the larva could not defend itself. Care was taken not to inflict any injury to the larva and the larvae injured were discarded. For prey densities beyond 16, the volume of the experimental jars was reduced instead of increasing the numbers of the prey, so that counting and replacing the larvae were done easily without any disturbance to the experiment.

2.2 *Procedure for application of 'disc' equation*

The various parameters followed in the 'disc' equation to describe the functional response of *E. furcellata* adults at different prey densities are listed below.

(i) x—Prey density.
(ii) y—Total number of prey killed in a given period of time, Tt.
(iii) x/y—The attack ratio.
(iv) Tt—Total time in days when prey was exposed to the predator.
(v) b—Time taken for handling each prey by the predator.
(vi) a—'Rate of discovery' per unit of searching time.

Usually the prey discovery was instantaneous with very little time being required. Although the parameter 'rate of discovery' (a) is infinite, the predator did spend some time in searching for the prey at lower prey density. Assuming that the predation is proportional to the prey density and to the time spent by the predator in searching prey (Ts) the expression of the relationship is:

$$y = a\,Ts\,x. \tag{1}$$

But time available for searching is not a constant. It is reduced from the total time (Tt) by the time spent handling the prey. If we presume that each prey requires a constant amount of time 'b' for consumption, then

$$Ts = Tt - by. \tag{2}$$

Substituting (2) in (1)

$$Y = a\,(Tt - by)\,x, \tag{3}$$

or

$$Y = Tt\,ax/1 + abx \qquad \text{(Holling 1959).} \tag{4}$$

3. **Results**

E. furcellata is an active predator and it attacks the prey in spite of violent resistances of the prey. Further, prey density and predator age alter the response of the predator

at all prey stages. In view of these factors, the functional response of the predator was studied with reference to the prey density, prey defence and predator age.

3.1 *Prey density*

The total time (Tt) during which the prey are exposed to predation was 20 days. The number of prey killed increase with the prey density upto $x = 32$, in the case of first to third instar prey stages but beyond $x = 32$, no further increase in 'y' was evident. Hence the predator has all the prey quantity it can feed upon without any search effort, when the prey density is above 32. On the contrary, the number of prey killed increased only up to the prey density of 16, in 4th and 5th instar prey stages (figure 1). The mean maximum predation of defenceless larvae by a predator in 20 days was 91, 68·6, 51·6, 42·2 and 34·4 from the 1st to the 5th instar prey respectively. Similarly, for free larvae the value was 89·9, 67·6, 44·6, 35·1 and 28·5 from the 1st to the 5th instar prey respectively, indicating the role of prey density and defence in influencing the functional response of the predator.

3.2 *Prey defence*

The maximum predation at highest prey density will be represented by 'K' and the value remains higher in the 1st instar than the 5th instar prey. In defenceless larvae, the attacking time is taken as 0 in terms of days and the time taken to feed varied from 0·020 to 0·089 days in the 1st to the 5th instars of the prey. The third component, in addition to attacking and feeding, viz. the interval between the time of completion of feeding and the time for the predator to attack again is estimated as, 'b' value—feeding time for all stages. The value of 'b' assumed to be constant for all prey densities. At prey densities below 32, the predator spent sometime searching its prey and therefore the searching time was calculated following the formula, $Ts = Tt -$
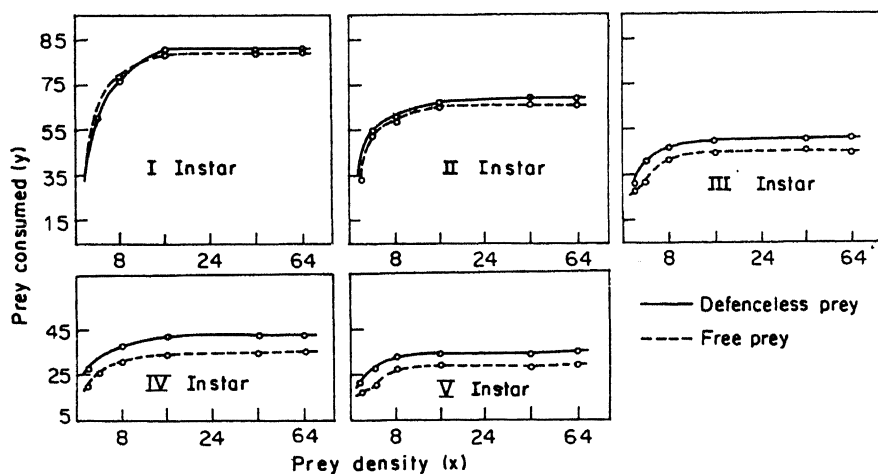
Figure 1. Functional response of *E. furcellata* adults to the density of *L. lepida*, when the prey is defenceless (D) and free to defend itself (F).

by in terms of days (table 1). Another parameter '*a*' the rate of discovery, was also estimated and the value was higher during the 1st instar stages than the 5th instar.

Unlike defenceless larvae the free larvae showed resistance especially during mature stage, hence the attacking time of the predator was calculated and the value was relatively negligible in the 1st and 2nd instar larvae. However, the predator took 0·00041, 0·0011, 0·0027 days to paralyse the 3rd to 5th instar stages of the prey. The '*b*' value of the predator increases from early to mature stages of the prey and from defenceless to free prey, and this is attributed to the following: (i) greater time is taken by the predator to paralyse the larger prey, (ii) increased amount of food consumption in a larger prey and (iii) longer time interval taken by the predator as resting period to attack another prey due to satiation (table 2). Similarly, the '*a*' value was reduced in free prey as compared with defenceless larvae and from early to mature prey and it could be due to 3 reasons: (i) '*a*' value is the proportion of prey attacked successfully per unit of searching time, (ii) continuous prey searching in view of the defensive activities of the larvae and (iii) predator search harder to find prey at smaller prey stages due to hunger.

3.3 *Predator age*

The importance of predator age in the functional response of the predator is quite obvious from the data provided in table 2. The values of '*K*', '*a*' and '*b*' have been calculated at two age intervals to illustrate the influence of predator age. Higher values in '*a*' and lower values in '*b*' was recorded during younger stages of the predator presumably due to active searching, quicker time taken to paralyse the prey and shorter time interval between successive attacks. In contrast, increased values in

Table 1. Summary of calculations used in fitting the functional response curves for defence-less prey (D) and free prey (F) for a first instar *L. lepida*.

Prey type	Prey Den. (x)	Prey att'd (y)	Max. y (K)	Days per y (b)	Days all y's (by)	Days searching (T_s)	Att. ratio (y/x)	$y/x \div T_s$ (a)
	1	18·6			4·09	15·91	18·6	1·17
	2	35·0			7·70	12·30	17·5	1·42
	4	61·4			13·51	6·49	15·3	2·37
D	8	78·8			17·32	2·66	9·9	3·70
	16	91·4			20·11	—	5·7	—
	32	90·6	91·0	0·22	19·93	—	2·8	—
	64	91·0			20·02	—	1·4	—
		$y = a(Tt - by)x = 2·17(20 - 0·22y)x$						2·17
	1	18·8			4·14	15·86	18·8	1·19
	2	35·6			7·83	12·17	17·8	1·46
	4	62·0			13·64	6·36	15·5	2·44
F	8	76·4			16·81	3·19	9·5	2·99
	16	89·4			19·67	—	5·5	—
	32	89·8	89·9	0·22	19·76	—	2·8	—
	64	90·4			19·89	—	1·4	—
		$y = 2·02(20 - 0·22y)x$						2·02

Total time 20 days.

Table 2. Functional response of *E. furcellata* adults on various stages of the prey, *L. lepida* at two different age levels.

Prey stage	Category D or F		K/T_t	b	a
		10 days	4·86	0·206	3·095
	D	20 days	4·55	0·220	2·174
I instar					
		10 days	4·90	0·204	2·491
	F	20 days	4·49	0·220	2·021
		10 days	3·88	0·258	3·256
	D	20 days	3·43	0·290	2·460
II instar					
		10 days	3·83	0·261	2·232
	F	20 days	3·38	0·300	2·560
		10 days	2·72	0·368	3·202
	D	20 days	2·58	0·390	3·520
III instar					
		10 days	2·39	0·419	2·091
	F	20 days	2·23	0·450	1·627
		10 days	2·26	0·443	1·740
	D	20 days	2·11	0·470	1·888
IV instar					
		10 days	1·99	0·501	1·366
	F	20 days	1·75	0·570	1·438
		10 days	1·87	0·534	2·493
	D	20 days	1·72	0·580	1·830
V instar					
		10 days	1·61	0·622	1·248
	F	20 days	1·43	0·700	1·090

'b' and decreased values in 'a' during older age of the predator is due to longer time interval between successive attacks and reduced searching ability.

3.4 Plant feeding

E. furcellata also feeds to some extent on plants and the frequency of plant feeding is higher at older age and during prey scarcity. The estimates of 'a' and 'b' values are slightly biased because of time spent on plant feeding has not been deducted from the time available for searching and further the process of plant feeding itself is not continuous. Supplementary plant feeding reduced the nymphal developmental period considerably so also highest percentage survival in nymphs. Plant diet alone proved insufficient for normal development and survival of the predator, but however plant feeding sustained the nymphs and adults for a considerable period during prey absence.

4. Discussion

Predators are always selective in the choice of their prey, from broad levels of preference for particular prey species to fine levels of preferred shapes, sizes, colours

and palatabilities (Law 1979). To describe and explain the functional response Holling (1959) proposed a 'disc' equation and successfully applied it to data on the following predator-prey systems: *Dohlbominus fulginosus* (Nees) vs. *Neodiprion sertifer* (Geoff.); (Burnett 1951, 1954); *D. fulginosus* vs. *Neodiprion lecontei* (Fitch) (Burnett 1958); *Chelonus taxanus* Cress. vs. *Anagasta kuhniella* (Zell.) (Ullyett 1949a); *Cryptus inornatus* Pratt vs. *Loxostege sticticalis* (L) (Ullyett 1949b); *Nasonia vitripennis* (Wlk.) vs. *Musca domestica* L. (Debach and Smith 1941). Mukerji and LeRoux (1969) explained the functional response of the predator *Podisus maculiventris* (Say) on the prey *Galleria mellonella* (L) in relation to its size of the prey and explained the positive applicability of Holling 'disc' equation. Morris (1963) also applied the equation to the predator *P. maculiventris* fed on larvae of *Hyphantria cunea* at different densities and confirmed its applicability within certain range of prey density and time. However in contrast to these, Haynes and Sisojevic (1966) were unable to apply 'disc' equation successfully to the data obtained on the predator *Philodromus rufus* in relation to the densities of the prey. In the present investigation the Holling 'disc' equation finds its applicability with reference to prey density and defence upto a range of prey density. The results indicate that the prey density is an important factor which modifies the rate of attack of the predator. Further, prey defence also plays a vital role in influencing the response of the predator at all prey densities and the rate of predation by *E. furcellata* decreases consistently with the age of the adult, presumably as a result of physiological changes associated with lesser food requirements.

E. furcellata draws moisture and nourishment from plants in addition to prey feeding, which possibly enable better survival rates at times of prey scarcity, since the ability of a predator to derive moisture and nourishment from plant materials is considered to be an adaptation that allows the predator to exploit the readily available resource without starving during prey scarcity (Sweet 1960). Plant feeding also helps highly mobile predators, providing food during migration in areas where the prey may not be abundant (Waddill nd Shepherd 1974). Further, plant feeding nature of predatory bugs increase the survival rate, reproductive capability and body size (Kiman and Yeargon 1985; Niranjo and Stimac 1985; Ruberson *et al* 1986). Stoner (1972) pointed out that the plant feeding nature of hemipteran predators would enable them to persist in agricultural habitats when prey becomes scarce. Hence, the supplementary plant feeding nature of *E. furcellata* is an adaptive habit for sustaining during the periods of prey scarcity or mobility in an agricultural habitat. Further studies on the individual chemical components of prey and foliage would enable us to device chemically defined artificial diets, so that mass rearing of the predator can be achieved.

Acknowledgement

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Role of endocrines in water balance in the coconut palm beetle *Oryctes rhinoceros*—Ultrastructural and electrophoretic studies

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Abstract. Ultrastructural observations on the brain median neurosecretory cells of the hydrated larvae (third instar) of *Oryctes rhinoceros* showed a well developed granulated endoplasmic reticulum and golgi bodies, indicating active state. The dehydrated animal on the other hand revealed inactive brain median neurosecretory cells with broken and swollen endoplasmic reticulum and underdeveloped golgi bodies. The axonal endings of the median neurosecretory cells in the corpus cardiacum of the dehydrated animal showed retention of secretory granules. Polyacrylamide gel electrophoretic studies also showed accumulation of neurosecretory materials in corpus cardiacum and brain samples of the dehydrated animal than those of the hydrated animal. The above results provide additional evidence that the brain median neurosecretory cells are involved in the regulation of water balance by secreting a diuretic hormone in response to hydration stress.

Keywords. Water balance; neurosecretory cells; diuretic hormone; ultrastructure; electrophoresis; *Oryctes rhinoceros*.

1. Introduction

The mechanism of hormonal regulation of water balance in insects is reviewed by Maddrell (1986). Histophysiological studies on the role of the brain median neurosecretory cells (MNSC) in water balance using light microscopy were studied in few insects (Nayar 1960; Wall and Ralph 1962; Highnam *et al* 1965; De Wilde 1966; Dogra and Ewen 1969; Jarial and Scudder 1971). In *Oryctes rhinoceros* light microscopic studies showed that the MNSC of the brain are concerned with secretion of a diuretic hormone (Kannan and Prabhu 1985), while the neurosecretory cells of the ventral nerve cord produce an antidiuretic principle (Kannan and Prabhu 1986) in relation to hydration and dehydration stresses respectively.

In the present study ultrastructural and electrophoretic methods were employed to verify our earlier observations regarding the role of the brain MNSC in hydration and dehydration stresses in the coconut palm beetle *Oryctes rhinoceros* (Coleoptera: Scarabaeidae).

2. Materials and methods

Third (final) instar larvae of the coconut palm beetle *O. rhinoceros* were used for the present study. Laboratory rearing methods and those used for inducing hydration and dehydration stresses in animals were described earlier (Kannan and Prabhu 1985).

2.1 *Electron microscopy*

The neuroendocrine complex consisting of brain, corpus cardiacum (CC) and corpus allatum of experimental animals were dissected out after 3 days of stress treatment, fixed in 3% gluteraldehyde in 0·1 M cacodylate buffer at pH 7·2 for 2 h, post-fixed in 1% osmium tetroxide for 1 h, dehydrated in acetone series and embedded in Araldite 502 resin. Semithin and ultrathin sections were cut with a LKB Nova ultra-microtome. Semithin sections were stained with methylene blue to locate neuro-secretory cells and ultrathin sections mounted in grids were stained with uranyl acetate and lead citrate. The stained grids were examined with a Hitachi trans-mission electron microscope at 75 KV.

2.2 *Electrophoresis*

For electrophoresis the method of Hames and Wood (1981) was followed. Poly-acrylamide slab gel (10%) containing anionic detergent sodium dodecyl sulphate (SDS) was used. Samples were prepared in dissociating sample buffer containing 2-mercaptoethanol and SDS; the final concentration being 5 pairs of CC or 5 brains per 50 μl of the sample buffer. Samples were run in discontinuous gel-buffer system and the gel was stained in 0·25% Coomassie blue 2R, destained and preserved in 7·5% acetic acid.

3. Results

3.1 *Electron microscopy*

In the present study MNSC of the brain were not classified into different types since each cell contains a heterogenous population of secretory granules of various sizes and nature. However the cells with identical characters namely the granules size and nature were taken to compare the effect of hydration and dehydration stress. The MNSC in the brain of the hydrated animal showed a very conspicuous nucleus with well defined double layered membrane and without any heterochromatic regions (figure 1). Endoplasmic reticulum (ER) was granulated, developed in plates and distributed in concentric arrays (figure 2). Golgi bodies (GB) were in abundance, arranged in elongated and curved stacks of cisternae and vesicles (figure 3). Membrane bound elementary neurosecretory granules were found in the perikarya. There were two types of electron dense granules differing in size with diameters 2100 and 1500 Å. Electron luscent granules of size 1200 Å were also present. Most of the granules were clustered in groups around the GB.

The brain-MNSC of the dehydrated animal also showed a prominent nucleus but the nuclear membrane was not well defined and comparatively more hetero-chromatin was present than that in the hydrated animal (figure 5). Cytoplasm is much compact. ER was found to contain less number of ribosomes. Most of the ER were broken and swollen into elongated vesicles like structures (figure 6). GB were few in number and not well organized. Elementary secretory granules were less in number which were of 3 types—electron dense granules of 2100 and 1500 Å diameter and an electron luscent type having a diameter of 1200 Å. In the perikarya of the
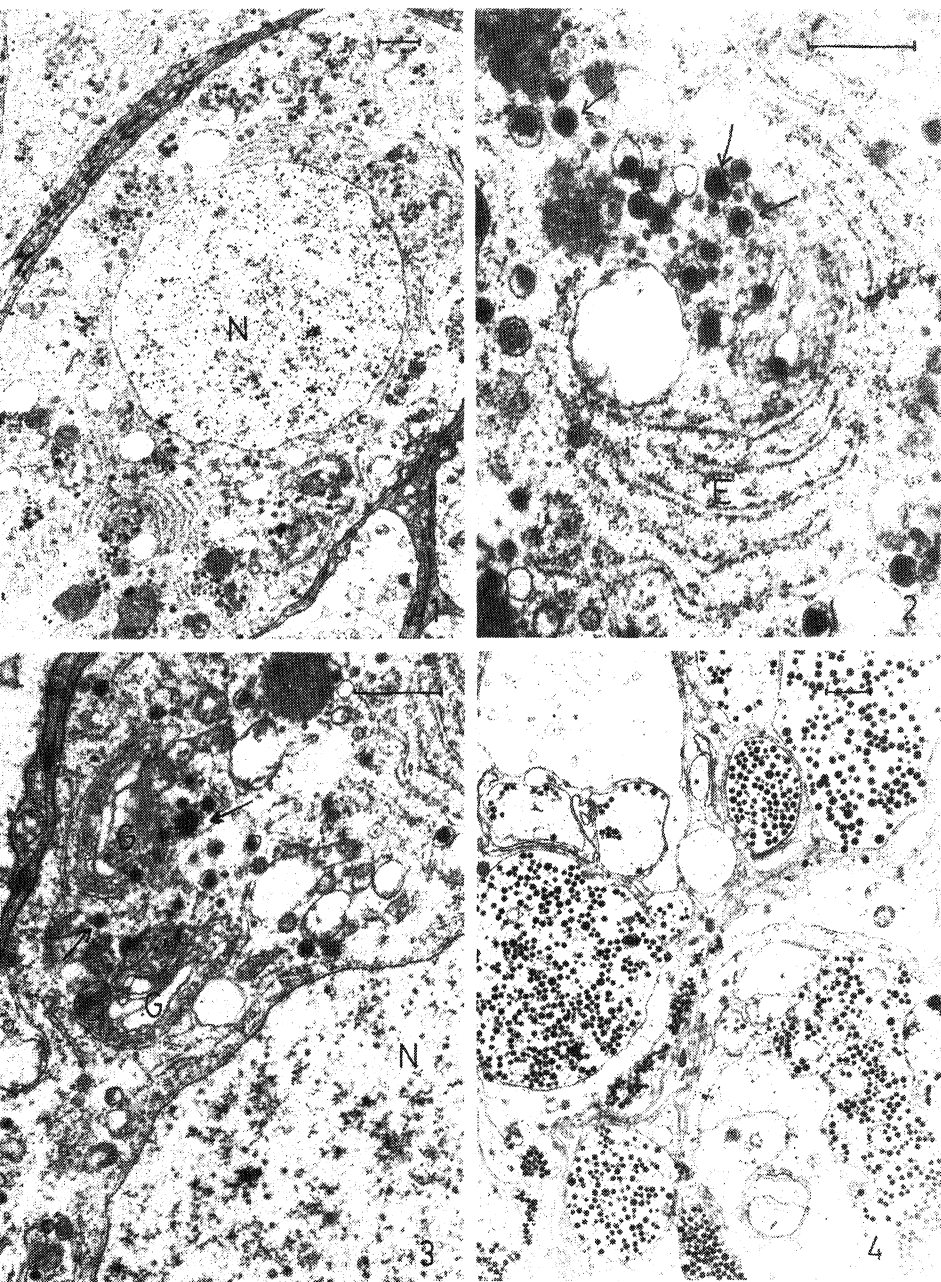
Figures 1–4. Electron micrographs of the brain-MNSC of the hydrated animal. **1.** Brain-MNSC of hydrated animal, low magnification. **2.** A portion of the brain-MNSC showing well developed rough ER and secretory granules (indicated by arrows). **3.** A portion of the brain-MNSC showing well developed GB. **4.** Axonal endings of brain-MNSC in the CC showing scanty granules (N, nucleus; E, endoplasmic reticulum; G, Golgi bodies). (Bar = 1 μm).
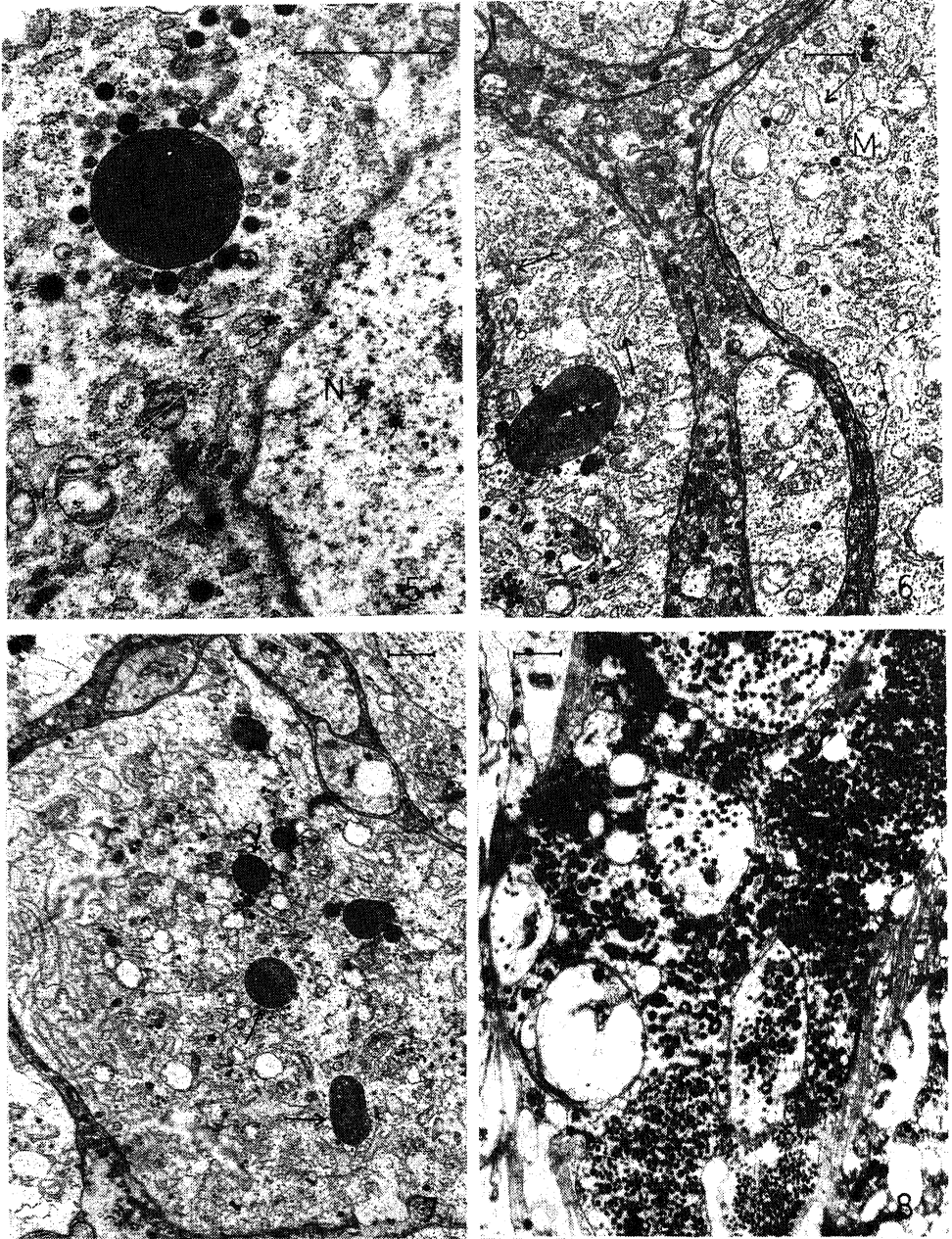
Figures 5–8. Electron micrographs of the brain-MNSC of the dehydrated animal. **5.** A portion of brain-MNSC showing nucleus and lysosomes surrounded by secretory granules. **6.** Brain-MNSCs showing swollen and broken ER (marked by arrows). **7.** Brain-MNSC with a number of lysosomes (marked by arrows). **8.** Axonal endings of the brain-MNSC in the CC showing dense accumulation of secretory granules (N, nucleus, L, lysosomes) (Bar = 1 μm).

brain-MNSC of the dehydrated animal a number of lysosomes were present (figure 7); several of them were found to be surrounded by elementary neurosecretory granules (figure 5).

The axonal endings of the brain-MNSC in the CC of hydrated animal were found to contain electron dense and electron luscent neurosecretory granules of the same size as in the perikarya (figure 4). However, the axonal endings of the dehydrated animals revealed increased number of neurosecretory granules. They also contained an additional type of large electron dense granules having a diameter of 2500 Å (figure 8).

3.2 *Electrophoresis*

The results of polyacrylamide gel electrophoresis (PAGE) revealed that the CC sample was characterized by 4 bands (figure 9). The band 3 showed a clear cut difference in the dehydrated animal, appearing much thicker when compared with hydrated sample. The brain sample contained more bands than that of the CC. In the brain sample of the dehydrated animal a band which showed electrophoretic
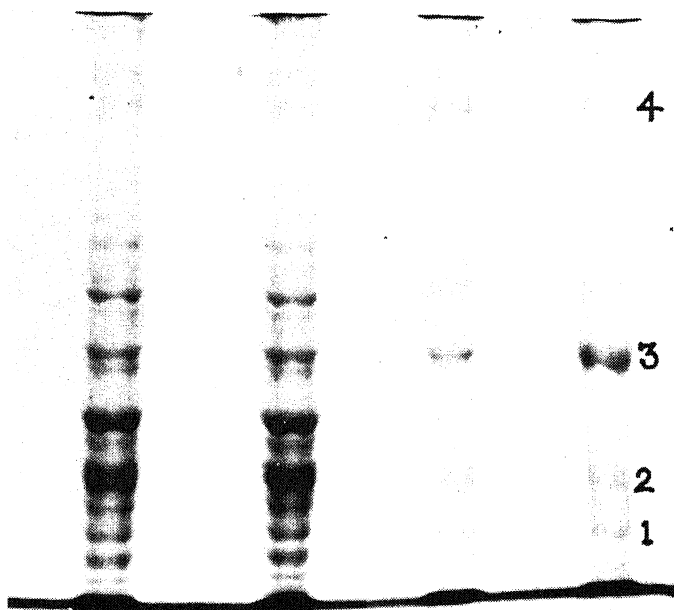
Figure 9. Electrophoreogram of CC and brain of hydrated and dehydrated animals (from left to right: brain of hydrated animal, brain of dehydrated animal, CC of hydrated and dehydrated animals). Band 3 is prominent in dehydrated brain and CC samples.

mobility identical to band 3 of the CC stained intensively than that of the hydrated animal. In both hydrated and dehydrated animals the CC band (no. 3) was thicker than its counterpart in the brain sample.

4. Discussion

The activity of a secretory cell can be determined by judging the nature of the

secretary machinery or endomembrane system of the cell consisting of ER and GB (Raabe 1982). There is no universally accepted criteria to measure the activity of a cell ultrastructurally, because they differ from cell to cell. Berlind (1977) gives a detailed account of active and inactive systems in neurosecretory cells. The presence of well developed profiles of ER tubules and GB made of stacks of cisternae and vesicles normally indicate high secretory activity. In the present study the brain-MNSC of the hydrated animal revealed well developed concentric arrays of elongated ER and GB composed of stacks of elongated cisternae and vesicles, indicating active secretory nature.

According to Berlind (1977) the criteria for the inactive state of a neurosecretory cell would include inconspicuous and ill defined ER, less number of ribosomes, under developed GB and large number of lysosomes. In the present study the brain-MNSC of the dehydrated animal showed broken and swollen ER with less number of ribosomes attached to it. The GB were not well developed and less in number. The abundance of lysosomes clearly indicated the inactive nature of the cells. The MNSC of *Calliphora erythrocephala* (Block *et al* 1966); *Bombyx mori* (Bassurmanova and Panov 1967); *Locusta migratoria* (Girardie and Girardie 1967); *Leptinotarsa decemlineata* (Schooneveld 1974) and *Achoea janata* (Muraleedharan and Vargheese 1986) were identified as active or inactive based on the above mentioned features.

Scharrer and Brown (1961), Geldiay and Edwards (1973) and Bell *et al* (1974) observed that inactive neurosecretory cells contained large number of secretory granules in the perikarya. But in the present study inactive cells i.e. the MNSC of the dehydrated animal contained less number of granules. This state may be due to the fact that lysosomes which are present in large numbers, take part in the removal of unwanted granules by a special type of autophagy known as crinophagy (De Robertis and De Robertis 1980). Rapid destruction of granules by lysosomes in inactive neurosecretory cells has been reported in *Locusta migratoria* (Girardie and Girardie 1967).

In the hydrated animal active release of granules account for the presence of only few granules at the axonal endings of the brain-MNSC in the CC. In the dehydrated animal the brain-MNSC may be inactive and will probably retain granules already synthesized as evidenced by the high accumulation of granules at the axonal endings. The axonal endings of the dehydrated animal contained an additional type of large electron dense granules which may be the secretory material required to overcome the hydration stress. Thus from the present study it appears that in *Oryctes rhinoceros* the MNSC of the brain synthesize and release a neurohormone in response to hydration stress. Tembrae (1979) also demonstrated ultrastructurally that neurosecretory cells of the thoracic ganglia of *Aeschna cyanea* have an active involvement in water and ionic balance in the haemolymph.

Electrophoretic results showed a thick band in the CC and brain samples of the dehydrated animal, indicating retention of granules already synthesised. Not only that this band (no. 3) was much prominent in the CC than in the brain. This observation suggests that the granules secreted in the perikarya are immediately transported to the axonal endings where they are temporarily stored.

The ultrastructural and electrophoretic findings thus support our earlier light microscopic findings (Kannan and Prabhu 1985), i.e. the brain-MNSC of *Oryctes rhinoceros* secrete a diuretic hormone, which is transported down the axon and stored in the CC for subsequent release into the haemolymph in response to hydration

stress. The target organ of the diuretic hormone is found to be Malpighian tubule (Madrell 1986). Bioassay studies using extracts of CC and brain under hydrated and dehydrated conditions are required for confirming these conclusions.

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Population structure of earthworms in woodlands of Karnataka

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Abstract. Woodlands in Karnataka differ with respect to earthworm species and their densities. *Lampito mauritii* Kinberg, *Pontoscolex corethrurus* Fr. Mull., and *Pheretima* (now, *Polypheretima*) *elongata* E Perrier are the most dominant species. Significant positive correlations were obtained between the species number, diversity and population density on one hand, and plant species number, abundance, diversity, cover and biomass on the other. A major factor for the variations in the richness of worms was accounted through vegetation-related factors. Soil organic matter had a strong influence on the worm diversity.

1. Introduction

The distribution of tropical earthworms is dependent on many edaphic and climatic factors (Dash and Patra 1977; Lavelle 1984; Habibullah and Ismail 1985; Krishnamoorthy 1986). It is known that the worms show preferences to leaf-converted organic matter, and that certain species exhibit leaf-specific habitat preferences (Krishnamoorthy 1986). Evidence has accumulated in recent years to show that plant diversity and density have significant effects on the distribution and activity of terrestrial invertebrate species (Rich *et al* 1983; Leather 1986; Perfecto *et al* 1986). Most of these studies were directed to understand the relationship of plant diversity and abundance of animal species. The role of above ground vegetation on species-richness, abundance and diversity of the worms are less understood. This paper deals with such a study and describes species-richness of some tropical earthworms in Karnataka. The species-richness indicates collectively the number of species existing in the area, their individual density and distribution.

2. Sites

The present study was carried out at 10 selected sites in Bangalore and Kolar Districts of Karnataka. Each study site was 2–3·5 ha in size and each one was 15–22 km away from the other. All the sites receive rains during July–September with an annual average of 415–590 mm. The study was undertaken during September 1986. The soil is a brown sandy loam.

3. Methods

Worm densities were measured by quadrat (1 M² each) random sampling. The extraction of worms was done by handsorting (Lewis and Taylor 1967). Plant

Table 1. Vegetation analysis of woodland sites.

Plant species	Density: Plants/1000 M² at site									
	1	2	3	4	5	6	7	8	9	10
Grasses										
Apluda aristata Linn	16				129		14	14	232	238
Bothriochloa pertusa Linn		19	9				39	41	278	571
Chrypsopogon fulvus Spreng						159		32	185	152
Cymbopogon nardus Linn				12				11	54	
Eragrostis aspora Jacq	26			11			94	24		
Hackelochloa granularis Linn					115	131				
Heteropogon contortus Linn	25				127		41		41	
Perotis indica Linn			12				72		147	
Saccharum spontaneum Linn		17								
Tripogodon pungeus Fisher					144	128		9	169	99
Herbs										
Caralluma umbellata Haworth				4		89	29	9		124
Cassia absus Linn				9				3		132
Cassia mimosides Linn		14			51				134	154
Crotalaria bifaria Linn			9		5	86		8		82
Euphorbia geniculata Ort		12					27		99	34
Indigofera glabra Linn		16					11	21		141
Phyllanthus asperulatus Hutch			8	8			13	4		
Rhynchosia refescers Willd					10	118			118	
Rothia indica Linn			11						144	
Sarxostemma acidum Roxb	8				5			7	37	
Tephresia tinctoria Pers	4				96	124	11			128
Tridax procumbens Linn	11								126	162

Table 1. (Contd.)

	1	2	3	4	5	6	7	8	9	10
Shrubs										
Ardisia solanacea Roxb							4		29	20
Caecelpinia decapetala Roth					10			9	92	29
Cassia hirsuta Linn				2	13					81
Celastrus paniculata Wild				7				13	54	24
Clausena dentata Wild					4					
Desmodium pulchellum Roth							8		44	28
Erythroxylum monogynum Roxb							4	12		29
Gymnesporia montana Roth							2			
Opuntia stricta Haworth						3	9		14	24
Phyllanthus polyphyllus Linn						24			58	26
Pterolobium hexapetalum Roth					14				91	
Toddalia asciatica Linn				6	3				59	24
Scutia circumscissa Linn					4					44
Xeromphis spinosa Thumb							5			18
Ziziphus oenoplia Linn						69		11	54	
Trees										
Anogeissus latifolia Roxb							1		4	4
Butea frondosa Linn							6		1	4
Memecylon umbellatus Bum					4		3	3	6	9
Tamarindus indica Linn						1	1			
Terminalia arjuna Roxb						6	4			
Vitex altissima Linn						2	7			4
Wrightia tinctoria Linn							2	3	8	23

abundance was assessed by counting the plants in an unit area. Plant and worm diversities were measured using Simpson-Yule index (*D*) (Southwood 1978). Plant cover was measured by Line-intercept method (Michael 1984), and the biomass (air-dried) was assessed according to Whittaker and Woodwell (1968). The soil organic matter was determined as loss-on-ignition of oven-dry soil over 24 h in a muffle furnace at 550°C (Allen *et al* 1974).

The relationships between variables (tables 4 and 5) were investigated. Analyses of variance, linear regression, multiple regression and principal components were followed according to Snedecor and Cochran (1967).

4. Results

The sites differed in plant species composition (table 1). The first 3 sites had only herbaceous vegetation, and the site number 4 lacked trees. The sites also differed in earthworm species composition and population densities (table 2). The dominance-order of species, however, differed with the sites (table 2). *Lampito mauritii* Kinberg, *Pontoscolex corethrurus* Fr. Mull., and *Pheretima* (= *Polypheretima*) *elongata* E Perrier were the most dominant and common species occurring in a majority of sites. *L. mauritii* was the most abundant among all the recorded species (table 2).

The sites differed in vegetation factors as well as in worm's richness factors (table 3). Species number, diversity and density of earthworms established significant positive correlations with the plant species number, plant abundance, cover, biomass and diversity (table 4). The number of worm species and those of plant species in the sites established a significant curvilinear relationship.

The worm species number, density and diversity were correlated to individual

Table 2. Earthworm species composition and abundance (as indicated by percentage of total number of worms found) at the selected sites of the study.

								Lm (32)	Lm (31)
						Lm (39)	Op (31)	Ga (21)	Pel (18)
						Pel (19)	Pc (24)	Pel (16)	Ga (15)
					Pc (44)	Op (12)	Ob (18)	Db (9)	Oo (11)
				Db (48)	Pel (23)	Oo (11)	Pel (10)	Op (8)	Db (9)
		Pel (51)	Pel (55)	Pel (22)	Lm (19)	Db (9)	Lm (8)	Oo (7)	Op (8)
	Lm (65)	Pc (28)	Pc (31)	Pc (18)	Db (9)	Pc (6)	Oo (5)	Pc (5)	Ob (6)
Pc (100)	Pc (35)	Lm (21)	Op (14)	Lm (12)	Oo (5)	Ob (4)	Db (4)	Ob (2)	Pc (2)
Site no: 1	2	3	4	5	6	7	8	9	10

Number in the parentheses indicate the percentage of individuals to the total number of worms found at the site. Lm, *Lampito mauritii* Kinberg; Pel, *Pheretima elongata* E. Perrier; Ga, *Griphidrillus annandale* Stephenson; Oo, *Ocnerodrilus occidentalis* Eisen; Db, *Drawida barwelli* Beddard; Op, *Octochaetoides pattoni* Beddard; Ob, *Octochaetoides beatrix* Beddard; Pc, *Pontoscolex corethrurus* Fr. Mull. Density of worms at the site is given in table 3.

Table 3. Earthworm species-richness in relation to plant abundance and diversity in the selected woodland sites of the study.

Site No.	Soil organic matter (%)	Number of earthworm species	Popu-lation density of worms no. per M³	Worm diversity	Number of plant species per ha.	Plant abundance no. per 1000 M²	Plant diversity (D)	Esti-mated % plant cover	Estimated above ground plant biomass* (tonnes per ha)
1	6·7	1	83	0	6	90	0·7930	20	0·25
2	7·2	2	98	0·4596	5	78	0·8056	20	0·50
3	8·9	3	110	0·6234	6	64	0·8077	30	0·75
4	9·8	3	132	0·5877	8	59	0·8397	45	1·20
5	11·9	4	176	0·6812	17	746	0·8626	45	2·00
6	10·6	5	192	0·7211	13	940	0·8778	50	7·00
7	11·3	7	214	0·7798	23	407	0·916	55	16·00
8	10·9	7	229	0·8014	18	234	0·8989	50	18·00
9	12·1	8	203	0·8137	26	2263	0·919	60	19·00
10	9·8	8	247	0·8267	29	2613	0·9292	60	21·00

*Air-dried.

Table 4. Linear regression coefficients for comparisons between worms richness factors and vegetation factors of biotopes.

Comparisons (x, y sequence)	Linear regression equation	Correlation coefficient (r)
Worm density and no. plant spp.	$y = 0·1463x - 9·7639$	0·9371**
Worm density and plant abundance	$y = 10·5337x - 1027·875$	0·6515*
Worm density and plant cover	$y = 3·24 + 0·2391x$	0·9253**
Worm density and plant biomass	$y = 0·1153x - 12·2482$	0·8861**
Worm density and soil organic matter	$y = 5·811 + 0·0244x$	0·7703**
Worm density and plant diversity	$y = 0·0523 + 0·0048x$	0·9755**
No. of worm species and no. of plant species	$\log y = 0·5238 + 0·1163x$	0·9546**
No. of worm species and plant abundance	$y = 220x - 312$	0·6001^{NS}
No. of worm species and plant cover	$y = 6·4094x + 12·7349$	0·9921**
No. of worm species and plant biomass	$y = 3·301x - 7·2748$	0·9612**
No. of worm species and plant diversity	$y = 0·3245 + 0·0934x$	0·8655**
No. of worm species and soil organic matter	$y = 7·3255 + 0·5409x$	0·7520*
Worm density and no. of worm species	$y = 0·0433x - 2·317$	0·9506**
Plant biomass and worm diversity	$y = 0·46 + 0·019x$	0·6833^{NS}
Plant cover and worm diversity	$y = 0·0229 + 0·0139x$	0·8418**
Plant diversity and worm diversity	$y = 3·98x - 2·8156$	0·6153^{NS}
Plant abundance and worm diversity	$y = 1018·33 - 0·5286x$	0·2535^{NS}
No. of plant species and worm diversity	$y = 0·3215 + 0·0206x$	0·7538*
Worm density and worm diversity	$y = 0·03106 + 0·0036x$	0·8405**
No. of worm species and worm diversity	$y = 0·238 + 0·082x$	0·8391**
Soil organic matter and worm diversity	$y = 0·1109x - 0·4704$	0·8206**

NS, Non-significant; *$P < 0·01$; **$P < 0·001$.

components like plant species number, plant abundance, cover, biomass and diversity, and soil organic matter content of the sites, subjecting the data for a component analysis (Snedecor and Cochran 1967). The proportion of variation of these components were assessed with reference to earthworm richness at the sites. All these

components accounted individually for more than 13% of the total variation in the earthworm richness at the site. Multiple regression analysis showed that 85·59% of the variation in the worm-richness was accounted for by all vegetation factors of the site. These analyses suggested that the vegetation characteristics or factors contributed a great deal to the richness of worms at the selected sites.

The vegetation characters of the sites were ranked according to the magnitude of the total standardized regression coefficients (TSRC) from significant characters of worm-richness (table 5). The rankings of TSRC indicated (table 5) the strong influence of soil organic matter content of the site, on the diversity of the earthworm species. Plant diversity, biomass, soil organic matter and plant species number also established a strong influence over the number of inhabiting earthworm species (table 5).

5. Discussion

Natality, mortality, immigration, emigration and repositioning within a habitat are so far known to affect the spacial distribution of many terrestrial organisms (Taylor and Taylor 1979). The present results show that the type of above ground vegetation affects the spacial distribution of sub-soil earthworm communities. The deciduous and fallen leaf litter of the sites, decompose under moist conditions and characterize the organic matter content of the sub-soils. The latter may thus exert influence over the spacial distribution of worm-species. The envisaged correlations and correspondence between the above ground vegetation and the sub-soil earthworm community are significant when judged through the data presented here. Such correlations are already known with reference to insects (Kennedy and Southwood

Table 5. Importance of worm's richness factors and vegetation factors of biotopes as indicated by TSRC.

Rank	Comparisons	TSRC
(1)	Soil organic matter and worm diversity	0·1806
(2)	Soil organic matter and worm species number	0·1607
(3)	Plant diversity and worm diversity	0·1415
(4)	Plant biomass and worm density	0·1275
(5)	Soil organic matter and worm density	0·1136
(6)	Plant diversity and worm species number	0·0913
(7)	Plant abundance and worm species number	0·0538
(8)	Plant species number and worm species number	0·0367
(9)	Plant cover and worm species number	0·0291
(10)	Plant cover and worm density	0·0237
(11)	Plant species number and worm diversity	0·0189
(12)	Plant cover and worm diversity	0·0083
(13)	Plant species number and worm density	0·0063
(14)	Plant biomass and worm density	0·0062
(15)	Plant biomass and worm species number	0·0008
(16)	Plant diversity and worm density	0·0006
(17)	Plant abundance and worm density	0·0003
(18)	Plant diversity and worm density	0·0001

1984), birds (MacArthur and MacArthur 1961), desert lizards (Pianka 1966) and desert rodents (Rosenzweig and Winakur 1969). Probably niche segregation, species diversity and vegetation type of the sites will contribute to the community and population structure of the sub-soil earthworms.

The results of the present study also reveal about the inter-specific earthworm associations. In the woodland sites studied, the megascolecids dominate in their population density and structure more than the ocnerodrillids and lumbricids (Rank correlation coefficient 0·7757). The dominance noticed has not affected their coexistence with other species and in a majority of sites the association is maintained well. Such associations were noticed earlier in the worms of forest soils of southern Sweden (Nordstrom and Rundgren 1973). Dominance and diversity of a group of earthworms differ with the site; probably due to the type of litter that the site constitutes which is mostly preferred by the species (Lavelle 1983). In our laboratory, it was found that *Lampito mauritii* prefers decomposing straw and *Pontoscolex corethrurus*, decomposing mango litter. Such dietary preferences are known that lumbricids resist the falling of forest trees in contrast to the megascolecids in Sweden (Nordstrom and Rundgren 1973). The reason for this could be the fact that megascolecids are more specialized in their diets.

Acknowledgements

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Effects of varying dietary protein level on the blood parameters of Cyprinus carpio

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Abstract. Effects of varying dietary protein levels, 14, 28, 38, 48 and 58%, on the blood parameters of juvenile *Cyprinus carpio* were studied. It was found that the various blood cells and related parameters respond differently to the variation in the dietary protein. Fishes fed with low (14%) and high (58%) level protein showed lesser values of red blood cells, haemoglobin content, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leucocyte count (TLC), differential leucocyte count (DLC) and coagulation time when compared with optimum protein level (38%). On the other hand, immature red blood cells, erythrocyte sedimentation rate (ESR) and mean corpuscular volume values of fishes fed with low (14%) and high (58%) level proteins were higher than that of the fish fed with optimum (38%) protein level.

Keywords. Dietary protein levels; *Cyprinus carpio*; haematology.

1. Introduction

Remarkable progress has been made over the past two decades in the study of dietary nutrient requirements of fishes (Halver 1972; Cowey and Sargent 1972, 1979; National Research Council 1981, 1983; Millikin 1982). The growth and health condition of fish is affected by nutritional factors like feeding frequency (Sampath and Pandian 1984), food quantity (Pandian and Raghuraman 1972) and food quality (Vivekanandan 1977). However, studies on the effect of dietary protein level on blood parameters are meagre. Piscine haematology is increasingly gaining significance due to its importance in the study of fish health under different conditions of life and environment, as elaborated by Hickey (1976) and Joshi *et al* (1980). Hence the present investigation is an attempt to relate the protein requirement of fish to the health condition with special reference to the blood parameters.

2. Materials and methods

Samples of *Cyprinus carpio* were collected from Manimuthar Dam and acclimatized to laboratory conditions for a period of 10 days. Healthy fishes (10 ± 1.23 g) were chosen from the stock and divided into 5 groups. They were fed with 5 levels of protein (14, 28, 38, 48 and 58%) in the diet. The experimental diets with different levels of protein (14, 28, 38, 48 and 58%) were prepared by square method of food compounding (Hardy 1980). The important ingredients used for the feed preparation were groundnut oil cake, rice bran, dried fish, dried silkworm pupae and tapioca flour. Since 38% is the optimum protein requirement for *Cyprinus carpio*, the group fed with 38% protein was treated as control group. Experiments were conducted in glass aquaria with 50 l capacity. Five fishes were reared in each aquarium. Three replicates were maintained for each group.

The test fishes were fed with weighed quantity of experimental feeds (3% of body weight) daily at 8 AM. After 2 h feeding period, unfed and faecal matters were removed, dried and weighed. The experiment was conducted for 190 days.

At the end of the experimental period the test fishes were sacrificed and blood was collected by severing the caudal peduncle by means of a standard micropipette (Johnsson-sjobeck and Larson 1978). Haematological tests were conducted by routine clinical methods (Wintrobe 1978). The red cell indices like mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to Johansson-Sjobeck and Larson (1978).

3. Results

The changes observed in blood parameters of *C. carpio* are given in table 1. The test fishes fed with normal level of protein (38%) showed the maximum total red blood cell (RBC) count and the number declined in both low and high levels of protein. The difference in RBC count between the fish fed with 38% protein and the lowest (14%) and the highest protein levels (58%) was statistically significant (38% vs 14%; $t = 3.550$ $P < 0.02$; 38% vs 58%; $t = 3.070$ $P < 0.02$). Similar trend was observed for TLC, thrombocyte, basophil, haemoglobin (Hb), haematocrit (Ht), MCH, MCHC values and coagulation time. On the other hand, immature RBC, ESR, MCV, lymphocyte and neutrophil values showed the opposite trend. Minimum number of immature RBC (2.69 ± 0.64/1000 cells) was noted in control fish whereas the maximum number (6.67 ± 0.71/1000 cells) was observed in fishes fed with low (14%) level protein. Similarly the maximum ESR (2.30 ± 0.63 mm^{-1}) and MCV (142.53 ± 1.19 fl) values were observed in fishes fed with low (14%) protein; these values in the control fish averaged to 1.29 ± 0.59 mm^{-1} and 113.04 ± 1.28 fl, respectively.

Table 1. Effects of varying dietary protein level on the blood parameters of *C. carpio*.

Blood parameters	Protein levels				
	14%	28%	38%	48%	58%
Total RBC ($\times 10^6$/mm^{-3})	2.33 ± 0.31	3.43 ± 0.42	3.58 ± 0.39	3.47 ± 0.33	2.45 ± 0.35
Immature RBC (per 1000 cells)	6.67 ± 0.71	2.89 ± 0.51	2.69 ± 0.64	2.99 ± 0.69	5.94 ± 0.73
Hb content (%)	5.21 ± 1.06	10.16 ± 1.22	10.73 ± 1.31	10.08 ± 1.22	6.53 ± 0.93
ESR (mm^{-1})	2.30 ± 0.63	1.36 ± 0.36	1.29 ± 0.59	1.38 ± 0.38	1.41 ± 0.61
Haematocrit PCV (%)	33.21 ± 1.23	40.32 ± 1.42	40.47 ± 1.31	40.21 ± 1.23	34.11 ± 1.49
MCV Ht/RBC $\times 10$ (fl)	142.53 ± 1.19	117.55 ± 1.13	113.04 ± 1.28	115.87 ± 1.25	139.22 ± 1.18
MCH Hb/RBC $\times 10$ (pg)	22.36 ± 1.15	29.62 ± 1.34	29.97 ± 1.12	29.04 ± 1.34	26.65 ± 1.15
MCHC Hb/Ht $\times 100$ (g/lt)	15.68 ± 1.04	25.19 ± 1.18	26.51 ± 1.42	25.06 ± 1.32	19.14 ± 1.19
Total white blood cells ($\times 10^3$/mm^{-3})	55.68 ± 1.03	61.21 ± 1.34	61.36 ± 1.03	60.45 ± 1.08	56.11 ± 0.98
Thrombocytes (%)	53.66 ± 1.43	54.67 ± 1.42	54.73 ± 1.08	54.18 ± 1.14	52.13 ± 1.06
Lymphocytes (%)	42.72 ± 1.09	39.97 ± 1.13	39.62 ± 1.07	39.98 ± 1.15	41.99 ± 1.23
Basophil (%)	37.21 ± 1.44	39.66 ± 1.61	41.98 ± 1.14	37.39 ± 1.23	37.31 ± 1.52
Neutrophil (%)	24.91 ± 0.92	23.68 ± 1.32	23.48 ± 1.62	23.52 ± 1.04	24.09 ± 1.43
Coagulation time (s)	22.07 ± 0.92	23.48 ± 1.12	23.69 ± 0.98	23.18 ± 1.32	22.99 ± 1.43

Each value is the average ($\bar{X} \pm SD$) of 3 observations.

4. Discussion

The present observations clearly show that protein levels in the diet affect the blood cells of *C. carpio*. A significant reduction of red cells was noted in test fishes fed with low (14%) and high (58%) levels of protein. This reduction may be caused either by the inhibition of erythropoiesis or by the destruction of red cells. An increase in the number of immature RBC indicates that the erythropoiesis is not affected by the protein variations. But the higher MCV value, on the other hand suggests that the anaemic condition may be due to the destruction of red cells. An increased MCV value in the present study may be considered as an index of RBC destruction leading to anaemia (Johnsson-Sjobeck and Larson 1979). The abnormalities observed in the mature and immature RBC in the present investigation are similar to the observations of Smith (1968) who has reported many abnormal RBCs with distorted nuclei in the population of matured and old cells in salmonids, carp and cat fishes exposed to the conditions of protein deficiency. This observation is also in accordance with the monochromic normocytic anaemia reported by Smith *et al* (1974) in rainbow trout *Salmo gairdnerii* exposed to pyridoxine deficient food.

A significant reduction in white cells was observed in test fishes fed with low protein. Similar decrease in white cells leading to leukopenia has been reported by Hawkins and Evans (1952) in rat and dog fed with vitamin deficient food (see also Agarwal and Mahajan 1982).

Even though the total leukocyte count showed a remarkable reduction in number in low protein fed fishes, the leukocyte types like lymphocytes and neutrophils showed a marked increase. The increase in lymphocytes may be due to the stimulated lymphopoiesis or due to an increased release of lymphocytes from lymphomycloid tissues (Ellis 1976). In the present investigation the increase in lymphocyte value may be the result of direct stimulation of lymphopoiesis caused by protein variations in the experimental diets.

In the case of mammals, the neutrophils are said to be responsible for phago-cytosis and disposal of foreign bodies or damaged tissues. McLeay and Brown (1974) have attributed a similar role for the neutrophils in fishes. According to them the elevated neutrophil count in fish is an index of tissue damage or the entry of foreign bodies into the blood stream. In the present investigation, the elevated neutrophil count may be the result of tissue damage caused by physiological stress due to protein deficiency.

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Laboratory studies on the life span, growth, fecundity and embryonic development of *Daphnia cephalata* King (Crustacea: Daphniidae)

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Abstract. Studies on *Daphnia cephalata*, relating to its longevity, instar duration, growth and fecundity have been made. Fifteen of the 22 individuals reared became primiparous at the fourth instar, the remaining at fifth. During growth, the total length, carapace length and carapace height increased by 2·95, 2·20 and 1·79 mm respectively and the growth curves for the 3 morphometric dimensions are of the same shape. About 200 eggs were produced during the life span of 52 days when the animals were reared at 27±2°C. The early embryogeny resembles other tropical daphnids but for differences in the developmental period in the brood chamber.

Keywords. Daphnia—life span; growth; fecundity; embryogenesis.

1. Introduction

The animal component of freshwater zooplankton is dominated by 3 major groups, namely, the rotifers, cladocerans and copepods. Reproduction in the first two groups is mainly by parthenogenesis and, the third is bisexual. The present paper reports on the biology of *Daphnia cephalata*, with reference to the number of juvenile and adult instars, changes in the morphometric dimensions during growth, reproductive potentials and embryogenesis. A knowledge of the life cycle of this cladoceran may help elucidating its role in secondary production in the freshwater environment.

2. Materials and methods

The animals were isolated from a mixed zooplankton collected from the Chekkanoorani pond, Madurai (long: 78° 8′ E; lat: 9° 56′ N), southern peninsular India. The stocks were reared in a plastic trough using filtered pond water as the medium and were acclimated to the laboratory temperature of 27±2°C. The animals thrived well under the laboratory conditions.

2.1 *Growth and fecundity*

Twenty two first instar individuals of the same clutch were reared, each in a separate beaker containing 100 ml of pond water filtered through a nylon mesh of about 150 μ. The minute phytoplankton and detritus passing through this mesh proved adequate for the normal growth and activities of *D. cephalata*. Daily observations were made on total length (TL), carapace length (CL), carapace height (CH), moults, number of eggs in the brood and the number of young ones released. For the first 3 observations the measurements were made using ocular micrometer. For life cycle studies, the method adopted for *Simocephalus acutirostratus* (Murugan and

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aboratory studies on the life span, growth, fecundity and embryonic evelopment of *Daphnia cephalata* King (Crustacea: Daphniidae)

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Abstract. Studies on *Daphnia cephalata*, relating to its longevity, instar duration, growth and fecundity have been made. Fifteen of the 22 individuals reared became primiparous at the fourth instar, the remaining at fifth. During growth, the total length, carapace length and carapace height increased by 2·95, 2·20 and 1·79 mm respectively and the growth curves for the 3 morphometric dimensions are of the same shape. About 200 eggs were produced during the life span of 52 days when the animals were reared at 27±2°C. The early embryogeny resembles other tropical daphnids but for differences in the developmental period in the brood chamber.

Keywords. Daphnia—life span; growth; fecundity; embryogenesis.

Introduction

he animal component of freshwater zooplankton is dominated by 3 major groups, amely, the rotifers, cladocerans and copepods. Reproduction in the first two groups mainly by parthenogenesis and, the third is bisexual. The present paper reports on ie biology of *Daphnia cephalata*, with reference to the number of juvenile and adult istars, changes in the morphometric dimensions during growth, reproductive otentials and embryogenesis. A knowledge of the life cycle of this cladoceran may elp elucidating its role in secondary production in the freshwater environment.

Materials and methods

he animals were isolated from a mixed zooplankton collected from the hekkanoorani pond, Madurai (long: 78° 8′ E; lat: 9° 56′ N), southern peninsular India. he stocks were reared in a plastic trough using filtered pond water as the medium nd were acclimated to the laboratory temperature of 27±2°C. The animals thrived ell under the laboratory conditions.

1 Growth and fecundity

wenty two first instar individuals of the same clutch were reared, each in a separate eaker containing 100 ml of pond water filtered through a nylon mesh of about 50 μ. The minute phytoplankton and detritus passing through this mesh proved dequate for the normal growth and activities of *D. cephalata*. Daily observations were made on total length (TL), carapace length (CL), carapace height (CH), moults, umber of eggs in the brood and the number of young ones released. For the first 3 bservations the measurements were made using ocular micrometer. For life cycle tudies, the method adopted for *Simocephalus acutirostratus* (Murugan and

Sivaramakrishnan 1973), *Moina micrura* (Murugan 1975a), *Ceriodaphnia cornuta* (Murugan 1975b) and *Leydigia acanthocercoides* (Murugan and Job 1982) was followed. However, observations were restricted only to the 15 individuals which became primiparous at 4th instar.

An attempt has been also made to study the size of the young ones released by successive broods. For this, random samples of 25 neonates released by successive broods were observed for TL, CL and CH. A similar method has been earlier followed on *Daphnia schodleri* by Lei and Clifford (1974).

2.2 *Embryonic stages in the brood chamber*

To study embryonic development in the brood chamber, individuals were reared separately. When recognizable stages of developmental significance within the brood were observed from the time of egg formation, the individuals were dissected under stereo microscope and the embryos isolated for photomicroscopy.

3. Results and discussion

3.1 *Preadult instar*

Of the 22 individuals reared, 15 became primiparous at the 4th instar and 7 at the 5th instar. The two categories had mean total lengths 0·9084 and 0·9035 mm respectively at their first instar, but in both, the mean total length of the primiparous females was nearly the same (table 1). Similar variations in the number of preadult instars have been reported in other cladocerans (Anderson and Jenkins 1942; Murugan and Sivaramakrishnan 1973; Murugan 1975a, b; Lei and Clifford 1974; Murugan and Job 1982). The present observations that individuals of larger initial size had fewer preadult instars and that the size of primiparous females was the same independent of the number of preadult instars, probably point to the influence of size on sexual maturity. Such a tendency has been reported earlier in *D. magna* (Green 1954, 1956). Anderson and Jenkins (1942) attribute variations in the number of preadult instars to hereditary and/or environmental factors such as differences in culture media.

3.2 *Growth curve*

The mean TL, CL, CH and duration of instars for the 15 individuals which became

Table 1. Mean total length of the pre-adult instars of *D. cephalata.*

Number of animals observed	Primiparous at instar	Instars				
		1	2	3	4	5
15	4	0·9084 (0·0183)	1·0666 (0·0264)	1·7976 (0·0459)	2·1679 (0·0464) Primiparous	
7	5	0·9035 (0·0506)	1·0696 (0·0872)	1·4785 (0·1857)	1·8339 (0·1408)	2·1767 (0·1370) Primiparous

Numbers in parentheses denote SD.

primiparous at 4th instar are presented in figure 1. The mean TL, CL and CH increased by 2·9487, 2·2019 and 1·7964 mm respectively during the life span of 24 instars in *D. cephalata*. The growth curves describe the same shape for the 3 morphometric dimensions. The maximum relative growth increment for all the 3 dimensions are between the 2nd and 3rd instars. The percentage survival of *D. cephalata* is graphically represented in figure 2.

Table 2 illustrates the percentage of net increase in the size of total length, carapace length and carapace height upto selected instars. The animals reach 42–45% (TL),
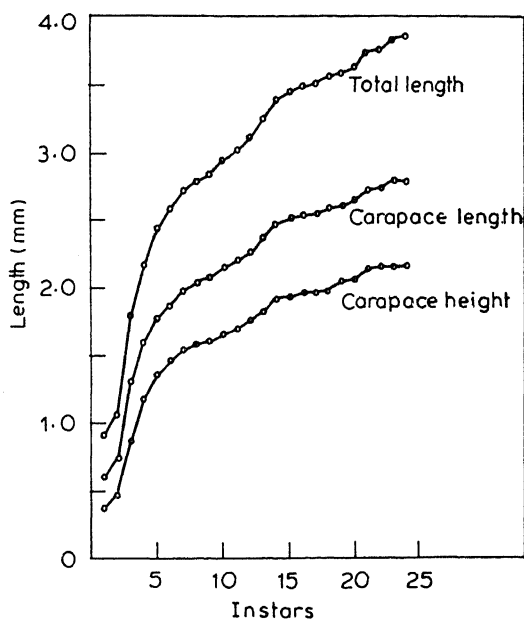
Figure 1. Growth curves for the 3 morphometric dimensions (total length, Carapace length and Carapace height) of *D. cephalata*.
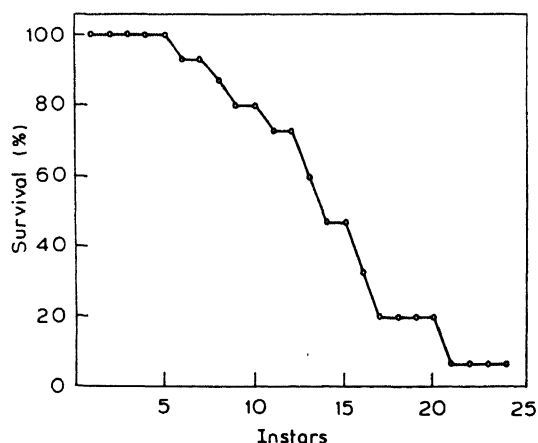
Figure 2. Survivalship curve of *D. cephalata*.

52–55% (CL) and 64–67% (CH) of their growth by 4th, 5th and 8th instars. Lei and Clifford (1974) have reported 65% growth in total length up to 8th instar in *D. schodleri* which is in agreement with the present value of 64·46%.

The total length, carapace length and carapace height are interrelated indices of the body size. Regression analyses were worked out for total length versus carapace length/carapace height (figure 3) and also carapace length versus carapace height (figure 4). In all cases the relationship is linear. For total length versus carapace length $Y = (0·7415)x − 0·0482$. For total length versus carapace height $Y = (0·6170)x − 0·1787$. For carapace length versus carapace height $Y = (0·8322)x − 0·1388$.

3.3 Fecundity

The birth rate of *D. cephalata* was studied on the basis of number of young ones released from the brood chamber. The number of young ones released were sometimes lesser than the number of eggs observed for a given brood which may be due to the production of non-viable eggs. The production of non-viable eggs was greater during the later instars. Further, the crowding of eggs within the brood made counting a difficult task. For these reasons the term 'young ones production' is suggested which is more appropriate than egg production. The mean number of young ones released per brood and age at each brood are presented in figure 5. The mean number of young

Table 2. Percentage of net increase in the size of total length, carapace length and carapace height upto selected instars for *D. cephalata*.

Instars	Percentage increase		
	TL	CL	CH
Upto 4	42·71	44·55	45·26
Upto 5	52·11	53·31	55·67
Upto 8	64·46	65·40	67·73
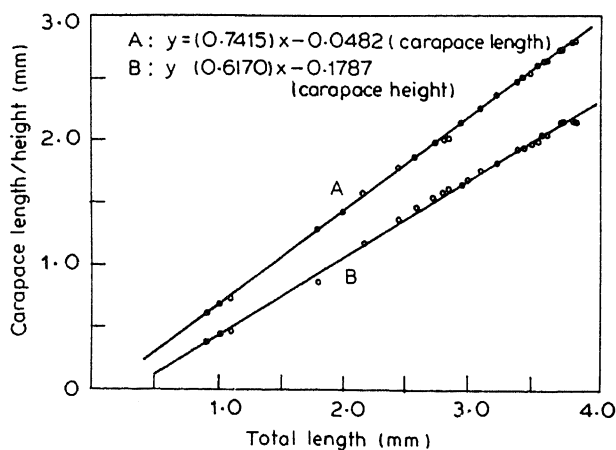
Figure 3. Regression analyses between total length and carapace length/carapace height in *D. cephalata*.
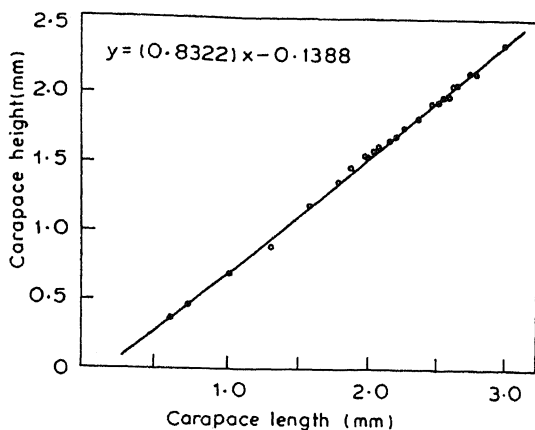
Figure 4. Regression analysis between carapace length and carapace height of *D. cephalata*.
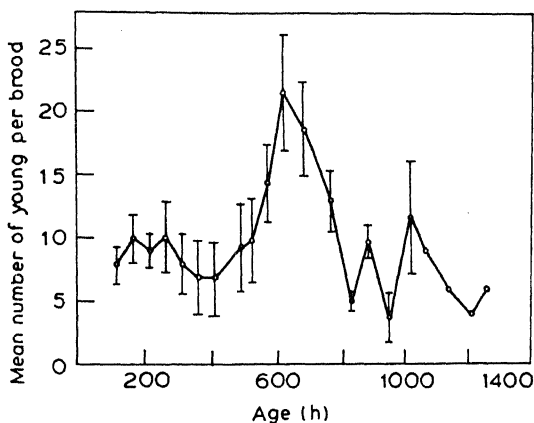
Figure 5. Age versus mean number of young per brood in *D. cephalata*.

ones released by the first brood (120 h) was 7·8 and from 408–620 h of age it increased from 6·75–21·5 following which there was a decrease till 826 h of age. The lowest number of 4 young ones was observed at an age of 1210 h. The mean number of young ones produced throughout the life span of 1258 h was 200·7. With a total life span of 1071, 557, 509 and 302 h respectively the tropical species of *Simocephalus acutirostratus, Leydigia acanthocercoides, Ceriodaphnia cornuta* and *Moina micrura* produce 248, 20, 123·6 and 61·18 eggs (Murugan and Sivaramakrishnan 1973; Murugan 1975a, b; Murugan and Job 1982).

The mean total length, carapace length and carapace height of the young ones released by successive broods are presented in figure 6. The 3 factors vary between 0·8143–0·9305, 0·5378–0·5968 and 0·3293–0·3657 nm respectively. In *D. schodleri*, with regard to total length, Lei and Clifford (1974) reported a tendency for the size of the young ones at each instar to increase progressively with increasing age. Green (1954) reported that the young ones released by the third brood were the largest in *D. magna*. In the present study the size of the young ones liberated increased upto the third brood.
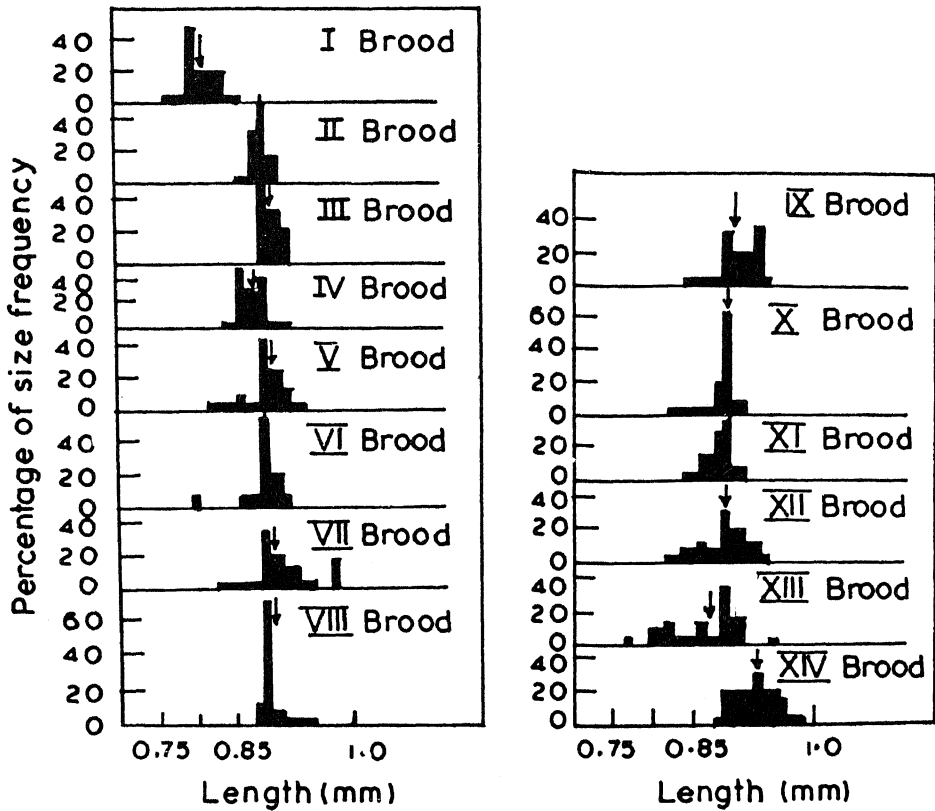
Figure 6. Relationship between the length and size of young liberated for successive brood in *D. cephalata*. Arrows indicate the mean length of the young.

3.4 *Development*

3.4a *Egg formation*: A pair of ovaries, one on each side of the alimentary canal, made their first appearance during the late third instar as elongated sacs. During the early fourth instar the contents of the ovaries were squeezed out in masses through the posterior openings of the ovary into the brood chamber. These masses became spherical and formed the eggs. Each egg, at the time of its formation encloses a central fat globule. The same mode of egg formation has been observed in *D. carinata* (Murugan and Venkataraman 1977) and *D. magna* (Green 1956).

Figure 7. **A.** Spherical egg. **B.** Elongated, headless embryo. **C.** Embryo with rudiment of the head lobe. **D.** Embryo with distinct head lobe and rudiments of appendages. **E.** Embryo with biramous antennae. **F.** Embryo with fused compound eye and ventrally curved caudal spine. **G.** First instar of *D. cephalata*. **H.** Third instar of *D. cephalata*. **I.** Primiparous individual.
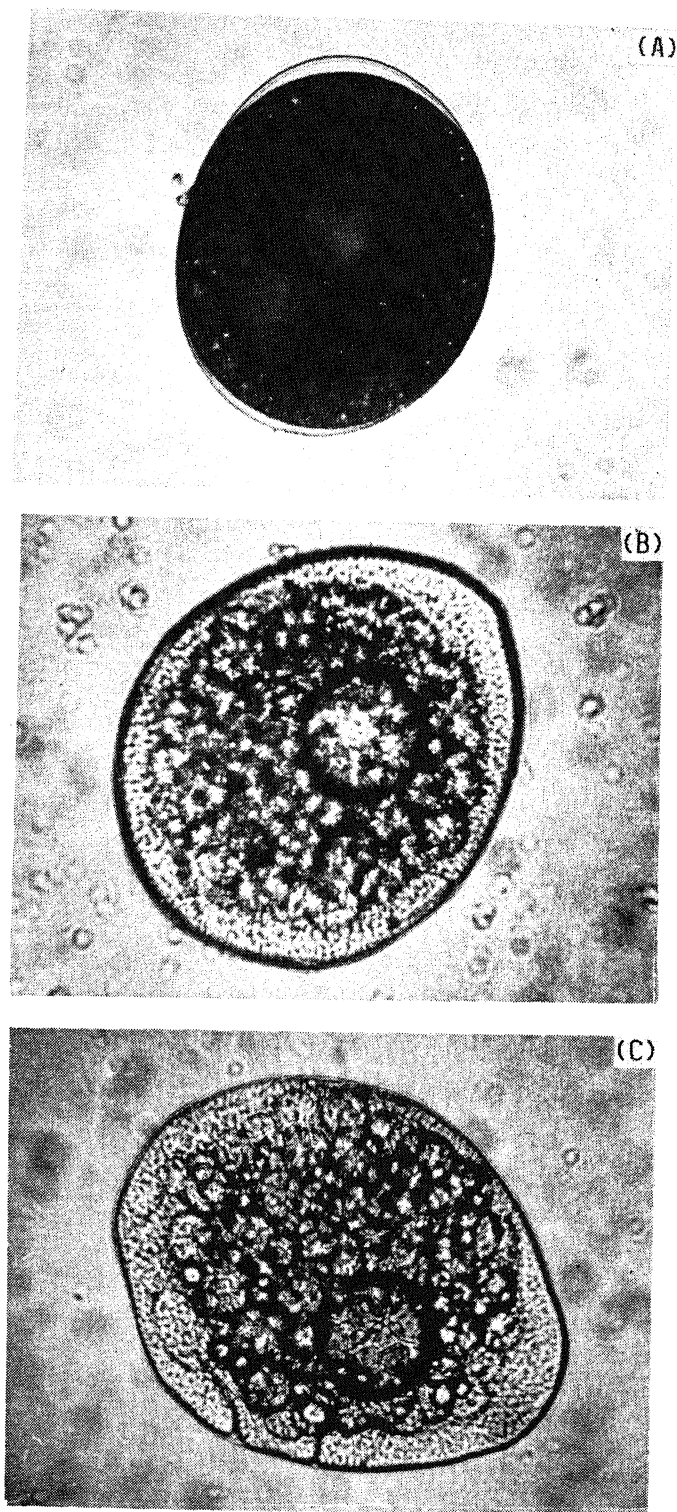
Figure 7.
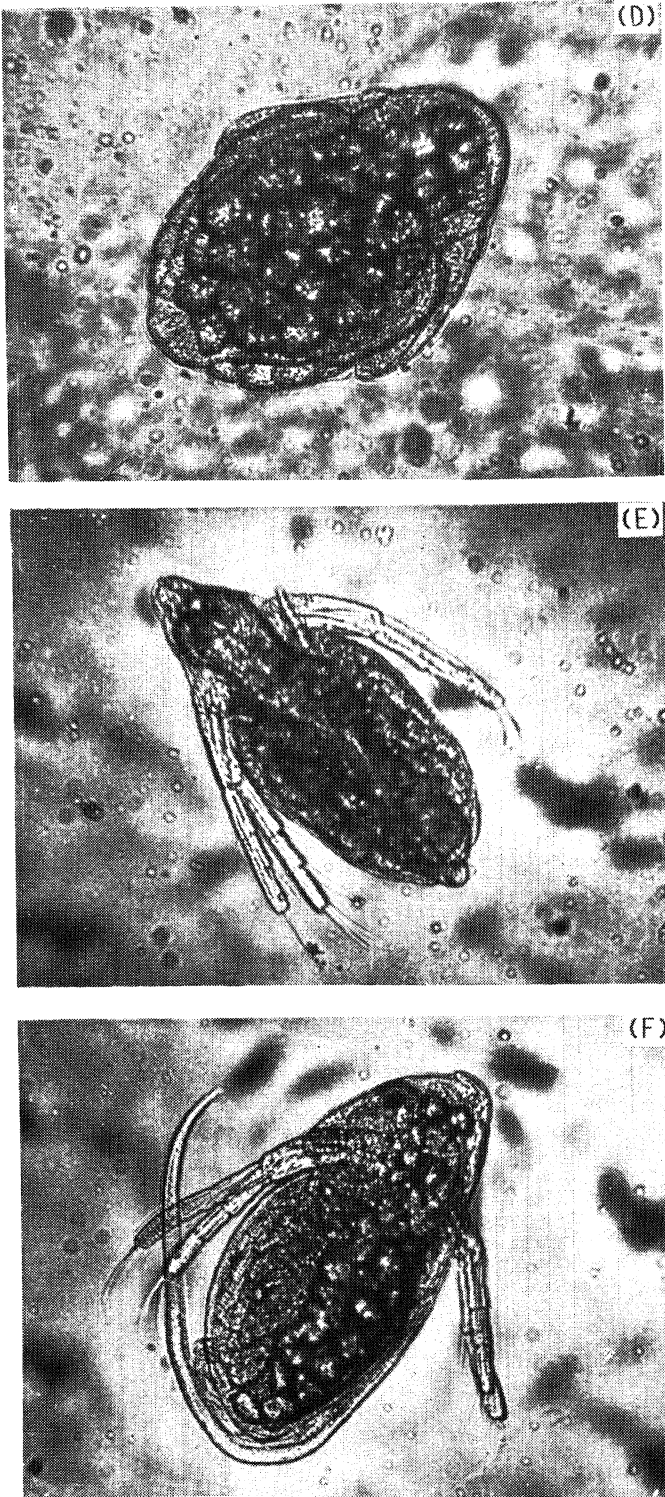
Figure 7. *Contd.*
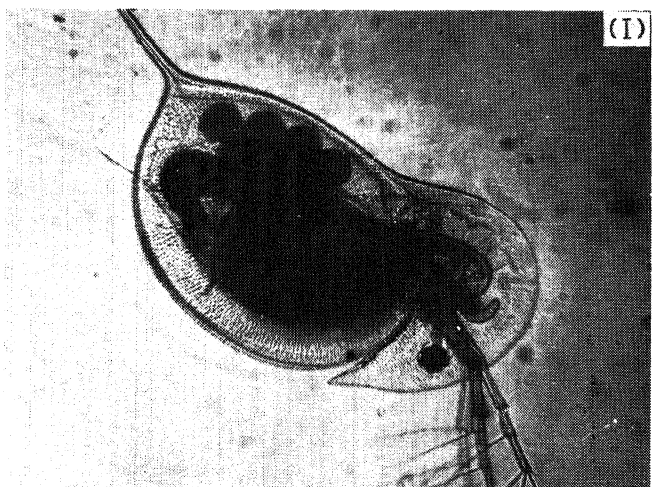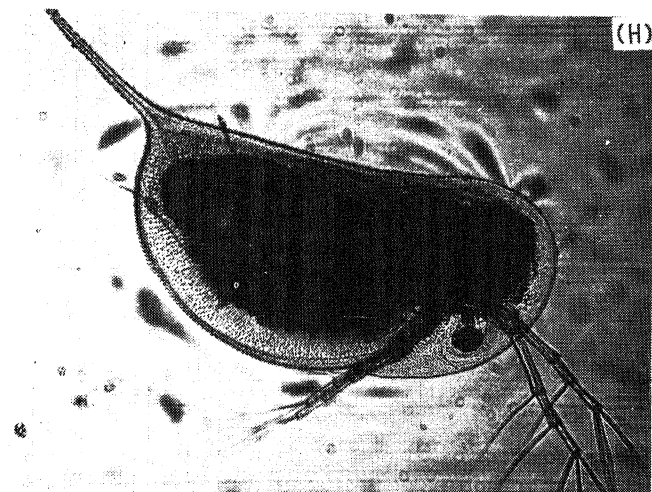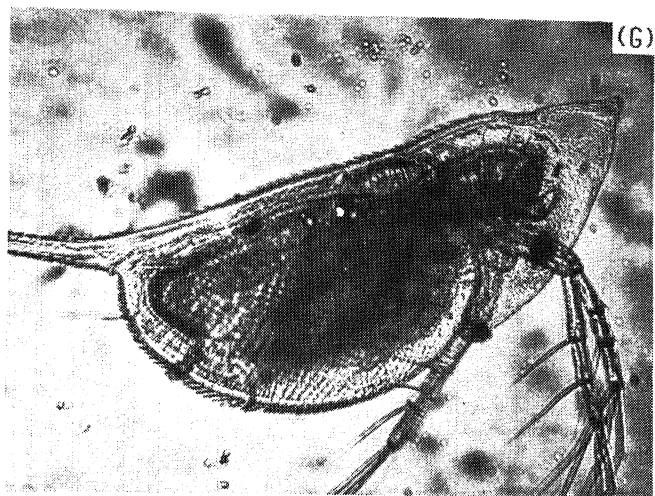
Figure 7. *Contd.*

3.4b   *Stages of embryonic development of D. cephalata:*

Stage 1:   The newly formed eggs were spherical with a mean diameter of 0·232 mm. Each egg was covered by an egg membrane with an inner opaque zone and a peripheral translucent zone (figure 7A).

Stage 2:   The egg became slightly elongated measuring 0·25 mm. The central fat globule was surrounded by cleaved cells. In addition to the outer egg membrane an inner naupliar membrane was seen (figure 7B).

Stage 3:   The embryo became oval measuring 0·267 mm long. The rudiment of the head lobe appeared. The cleaved cells along the periphery were enlarged (figure 7C).

Stage 4:   The elongated embryo measuring 0·375 mm, had well developed head lobe and antennae which reached nearly half the body length. Cleaved cells were visible in the cephalic region. The naupliar membrane was intact (figure 7D).

Stage 5:   The embryo measured 0·482 mm. It had well developed eye, functional biramous antennae with terminal and lateral spines and a posterior ventrally curved caudal spine. Rudiments of appendages were seen towards the posterior lateral margin. Cleaved cells were visible on the dorsal side. The tip of the rostrum was curved like a hook. Naupliar membrane bursts. The foregut made its appearance during this stage (figure 7E, F).

Stage 6:   This stage was represented by the first instar individuals on their release. The TL, CL and CH were 0·90, 0·60 and 0·36 mm respectively. The tip of the rostrum was pointed and straight. The characteristic helmet of *D. cephalata* was not formed yet. Eyes appear darker, heart pulsating and the alimentary canal was fully developed and the post abdomen with its spines. All the appendages were functional and the caudal spine was straight (figure 7G). The dorsal and ventral margins of the caudal spine were provided with short spines. The third and primiparous instars are shown in figure 7H, I.

The stages of embryonic development of *D. cephalata* are compared with allied temperate and tropical daphnids. There is a close similarity in the general developmental pattern though the duration of embryonic period varied. The present species completes development within 48 h (27°C) whereas a period of 70 h (22°C) is needed for *D. magna* (temperate), about 48 h (29°C) for *S. acutirostratus*, 40 h (29°C) for *L. acanthocercoides* and 24 h (29°C) for *C. cornuta, M. micrura* and *S. kingi* (tropical). These differences in developmental time may be due to variations in both ambient temperature and species specificity. As pointed out by Hutchinson (1967) the duration of brooding period while the young ones are still in the pouch, depends on the length of the instar.

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Nitrogen excretion in insects

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Abstract. As a result of excessive consumption of nitrogen more than necessary for their normal life processes, insects eliminate the excess quantities in one form or the other lest the ensuing toxicity should prove fatal to them. Voiding of several other nitrogenous compounds by insects besides uric acid via one common opening—the rectum, has posed difficulties in determining their nature as true excretory products or fecal nitrogenous waste matter. In the present review article an attempt has been made to present an almost up to date knowledge on the topic based on an intense search of literature which also includes the findings from the author's laboratory.

Keywords. Nitrogen excretion; insects; uricotelic pathway; uricolytic pathway; nucleicolytic pathway; *de novo* synthetic pathway; pterines; pteridines; tryptophanommochrome pathway.

1. Introduction

Extensive research has been carried out in the field of nitrogen excretion in insects and quite a few reviews on the subject have also appeared (Wigglesworth 1950; Prosser 1952; Roeder 1953; Craig 1960; Stobbart and Shaw 1974; Bursell 1967; Cochran 1975, 1984). Unfortunately however, many a lacuna still remains unexplored and unexplained leaving several gaps still unbridged. For example, ammonia formation, biogenesis of urea, the mechanism of uric acid/urate transport, the exact mode of uric acid biosynthesis, the operation or otherwise of Krebs-Henseleit urea cycle and the exact role of several nitrogenous compounds excreted etc., are some glaring posers which still remain unequivocally elucidated. Numerous evidences substantiated with experimental data in the field, are discordant and contradictory.

Occurrence of uric acid in insects has been known for more than 150 years (Wigglesworth 1972) and it is now well established that it constitutes about 80% of the excreted nitrogen which substantiates that uric acid is the main nitrogenous excretory substance of most terrestrial insects (Wigglesworth 1953; Terzian *et al* 1957; Irreverre and Terzian 1959; Hudson *et al* 1959; Razet 1961). This phenomenon had earlier induced Needham (1950) to term insects in general, as uricotelic. As a matter of fact, the basic concepts of excretory nitrogen metabolism were first established in the 1930s when animals were generally classified on the basis of the nitrogenous components which predominated in their excreta (Campbell 1973). While uricotelism was looked upon as an adaptation of insects to their terrestrial mode of life, the excretion of other toxic nitrogenous end products like ammonia and the extremely water-soluble urea was considered as a result of water shortage (Craig 1960; Stobbart and Shaw 1974). In recent years however, the enormous accumulating data evincing the presence of several other nitrogenous end products in the excreta of a variety of insects other than uric acid, have given a rude shock to the concept of uricotelism and probably necessitate its reassessment and modifications.

The detection of allantoin, allantoic acid and/or uric acid in the excreta of several

insects induced Bursell (1970) to broaden the term uricotelism in insects by including allantoin and allantoic acid along with uric acid or a mixture of any of the 3 compounds due to their biochemical relationship. This concept was further broadened by inclusion of hypoxanthine and xanthine excreted by several organisms due to the absence or modification of xanthine dehydrogenase activity in them (Tamura and Sakate 1975). Additional inclusions of compounds under uricotelism as suggested by Cochran (1975) are, adenosine (Lafont 1974), guanine (Mitlin and Vickers 1964; Descimon 1971) and uric acid riboside (Krzyzanowska and Niemierko 1980).

Uric acid on account of its physico-chemical properties has been considered as an almost ideal compound in insect excretory system. In the first place, its sparingly soluble property is well suited to it as an excretory product particularly, in a system where water retention is important. It also contains an appreciably high percentage of nitrogen, is highly oxidized and is capable of being eliminated in a dry pellet form. Although synthesized mainly in the fat body, the process of excretion in insects is carried out by the Malpighian tubule-rectum complex the structure and functions whereof have extensively been reviewed by Cochran (1975), Maddrell (1971, 1977, 1981) and Wessing and Eichelberg (1978).

Knowledge regarding the process of excretion and transport mechanism of uric acid is miserably scant. Nevertheless, based on its properties, several modes of their transport have been proposed. Firstly, on account of its ability to form urates, it occurs in the excretory system as the monosubstituted soluble salts of Na^+ or K^+ (Seegmiller 1969) and gets precipitated as the free acid subsequent to reabsorption of the purine base and water, the site of precipitation being the rectum. In *Rhodnius prolixus* however, the precipitation of uric acid occurs in the proximal portion of the Malpighian tubule (Wigglesworth 1931). The continuous recycling of base and water through the excretory system gradually results in a large accumulation of uric acid in the rectum of several insects. McNabb and McNabb (1980) have demonstrated that artificial buffered media containing Na^+ or/and K^+ are capable of maintaining high level of uric acid solubility for appreciably long periods whereas media containing NH_4^+, Ca^{2+} or Mg^{2+} rapidly reduce it to extremely low concentrations (Porter 1963a).

The presence of proteins in biological fluids is also known to enhance their solubility limits (Seegmiller 1969) and uric acid is known to form supersaturated solutions in such media. These solutions are quite stable and form even more stable lyophobic colloids (Porter 1963b). Although the transport of these stable colloids via haemolymph may be doubtful, their flocculation involving a sudden change in the pH or ion concentration or contact with urate crystals either in the Malpighian tubule or in the vicinity of its outer membrane, could possibly influence and facilitate the movement of uric acid and urates into the cells of the Malpighian tubule for elimination.

Influence of pH on the solubility of uric acid and urates is well known (Porter 1963a, b; McNabb and McNabb 1980). The increase in pH up to 9, increases the solubility of these two metabolites in an artificial medium while its powering reduces their solubility significantly. Haemolymp pH varies in different species of insects but it generally lies between 6·4–6·8 (Wyatt 1961). Although this apparently is not in favour of uric acid transport via haemolymph, it is likely that the surrounding pH gradients facilitate changes favouring the solubility of uric acid and urates.

Several investigators believe that urate transport in insects is under hormonal control (Bodenstein 1953; Milburn 1966; Thomas and Nation 1966). Isolation of 3-ribosyl-uric acid from insect tissues (Heller and Jezewska 1960; Jezewska *et al* 1967; Tojo and Hirano 1968) suggested this compound to play a transport role. Likewise, protein-bound urates have also been suggested as a means of transport of uric acid in insects (Hopkins and Lofgren 1968; Whitmore and Gilbert 1972; Farrell *et al* 1975). Thus it becomes quite clear that the insoluble nature of uric acid does not at all stand in the way of its internal transport in the excretory system of insects. At the same time, it is also not certain which particular mechanism they adopt in voiding it.

Considerable research has been carried out by several investigators on uric acid excretion in cockroaches and recounting them here with interesting findings will not be out of place.

Periplaneta americana although considered for a very long time by numerous authors (Nation and Patton 1961; Srivastava and Gupta 1961; McEnroe 1966; Bursell 1967; Corrigan 1970) as a uricotelic insect, a re-examination by Mullins (1971) and Mullins and Cochran (1972), led them to conclude the case to be different. Thin-layer chromatography and enzymatic spectrophotometry revealed that no uric acid was excreted by them. This was subsequently confirmed by Cochran (1973) on 20 species of cockroaches when even feeding of 91% casein diet did not induce the insects to excrete any uric acid or its metabolites allantoin, allantoic acid, urea or any of the other common excretory constituents like the purines, pyrimidines or pteridines. This led them to conclude that this group of cockroaches could not be considered in the classical sense as uricotelic and that they had different modes of dealing with excess dietary nitrogen.

P. americana does not excrete uric acid externally but appreciable quantities are synthesized and internally stored. Interestingly, it is also true that with the increase in the dietary nitrogen level, a corresponding increase in internal nitrogen storage also takes place in the form of urates (Haydak 1953; McEnroe 1956; Mullins 1974; Mullins and Cochran 1972, 1974, 1975a, b). During dietary stress under low nitrogen diet this stored uric acid is metabolized probably aided by the fat body symbionts and the nitrogen is utilized by the insects to maintain their nitrogen balance (Donnellan and Kilby 1967; Mullins and Cochran 1975b).

Mullins and Cochran (1976) examined nitrogen cation excretion and storage in 23 cockroach species representing 11 subfamilies and noted a significant correlation between body nitrogen and body uric acid nitrogen. Some species recorded very high levels of uric acid/urates amounting to 72% of their total body nitrogen whereas others in contradistinction, recorded low levels of only 30% of their body nitrogen. A less significant correlation was also observed between internal nitrogen storage and externally excreted nitrogen. Consequently, based on these and their earlier findings, Mullins and Cochran (1972) and Cochran (1973) concluded that the species that excreted about 62% of their fecal nitrogen as uric acid nitrogen, could be classified as uricotelic while those that excreted 70% as ammonia nitrogen, as ammonotelic. The significance of ammonia excretion in cockroaches is not yet clear but all cockroach species are known to excrete ammonia in varying quantities. *P. americana* which excretes comparatively more ammonia than any other species of cockroaches, releases very little of it from respiratory surface but mostly voids it via fecal pellets (Mullins 1974).

Accumulation of uric acid in general (Haydak 1953) and in the *Blattidae* in particular (Srivastava and Gupta 1961), has been demonstrated depending on the

dietary protein intake by the insects. This was subsequently confirmed by several investigators (Mullins and Cochran 1972, 1974, 1975a, b; Cochran 1976, 1979, 1981; Cochran *et al* 1979; Mainguet and Le Berre 1973; Mauchamp and Lafont 1975; Lafont and Pennetier 1975). Uric acid mobilization in American cockroaches led Ludwig (1954) and Ross (1959) to suggest uric acid as a nitrogen reserve for synthetic purposes under some endocrine control mechanism (Bodenstein 1953). Cockroaches while maintained on protein-rich diets, have been noted to mobilize in the fat body large quantities of uric acid without eliminating it externally (Cochran *et al* 1979; Cochran 1981). Likewise, Bursell's examination (1965a, b) of the excretory products of *Glossina morsitans* (tsetse fly) showed uric acid as the main product constituting about 70% of the total dry fecal matter excreted. Haematin was another component excreted in substantial amounts. In addition, Bursell also isolated a brightly fluorescent yellow pigment which is yet to be identified. The partially purified substance tallied with some of the properties of some pteridines that occur in insect excreta but also differed in many others. In addition, small quantities of urea, ammonia and a number of amino acids other than arginine and histidine were also detected.

Roth and Dateo (1964, 1965) demonstrated some species of cockroaches to employ their sex glands as a reservoir for uric acid deposition in addition to their function as accessory excretory organs when they poured the stored uric acid over the spermathecae during the process of copulation. Although this process of eliminating uric acid by the male cockroach during mating is not clear, Roth (1967) has suggested that it perhaps protects the spermatophore from being eaten by the female. Mullins and Keil (1980) have forwarded other explanations for the voiding of uric acid via the uricose glands.

During their metabolic studies and nitrogen excretion in metamorphosing and ovipositing sawfly *Neodiprion sertifer*, Fogal and Kwain (1974) observed a gradual increase in uric acid content in the female insects all through pupal development followed by a decrease at the adult stage as well as during egg production whereas in the male adults the accumulation continued in increased quantities. Since the uric acid depletion in the females could not be explained by the amount of allantoin, allantoic acid, urea or urea riboside (Jezewska *et al* 1967), it was speculated that it gets utilized to supply the nitrogen requirement for egg production.

The major part of uric acid in the non-pharate adult female of *N. sertifer* was found located in the meconium of the insect which on analysis, revealed the main bulk of the excreted nitrogen to be of uric acid origin along with small quantities of ammonia, urea, allantoic acid and the conspicuous absence of allantoin.

Analysis of the meconial fluid (material spurted out immediately after moth emergence) of two lepidopterans *Philosamia ricini* and the tasar silkworm *Antheraea mylitta* as reported by Pant *et al* (1984), revealed large quantities of uric acid and urea but without any trace of allantoic acid or allantoicase activity.

Studies of Mauchamp and Lafont (1975) on *Pieris brassicae* showed that uric acid and allantoic acid are the main excretory components. These authors also confirmed the earlier findings that most of these excretory products in the young developing larvae are located in the integuments associated with protein granules (Tsujita and Sakurai 1964, 1966, 1967; Barbier 1972). The integuments also contain ommochromes which get excreted before the insect goes into moulting as red pellets rich in uric acid but poor in allantoic acid, the former increasing 4-fold with the growth of the larvae. In this insect, the excreta are also characterized by a ratio of allantoic acid to

uric acid less than one due to low uricase activity. By the fifth instar stadium, most of the excreta are expelled and based on this, it was speculated that the larva of *P. brassicae* excretes about twice as much uric acid as allantoic acid, an observation, which is contrary to Razet's (1961) finding. However, different diets afforded different results evincing the influence of food composition on excretion of insects. Earlier, the same was emphasized by Mainguet and Le Berre (1973) in *Locusta migratoria* where diets play an important role in the excretion of uric acid and allantoic acid as well as on the secretion of the enzyme allantoicase and its activation.

Among insects, it is a point of interest to note that different species of insects belonging to the same or different order excrete both qualitatively and quantitatively different nitrogenous excretory products. For instance, the presence of purines other than uric acid is rarely detected in insect excreta and yet detection of hypoxanthine and xanthine identified in the excreta of *Melophagus ovinus* (Nelson 1958), *D. melanogaster* (Kursteiner 1961) and *Galleria mellonella* (Nation 1963), probably reflects peculiarities of purine metabolism traceable perhaps to genetic factors. Again, it is only the mutant of *D. melanogaster* lacking in the enzyme xanthine oxidase (Forrest *et al* 1956) that has been noted to excrete xanthine and hypoxanthine whereas the absence of guanase perhaps accounts for the presence of guanine in the excreta of the boll weevil *Anthonomus grandis* (Mitlin *et al* 1964).

Razet (1954, 1956,1961) demonstrated the presence of uric acid degradation products in insect excreta and suggested that the excretion of allantoin and to a lesser extent that of allantoic acid could be more general than believed to occur. As a matter of fact, all the enzymes participating in the stepwise degradation of uric acid to allantoin and allantoic acid have been found widely distributed in insects (Leifert 1935; Brown 1938a; Duchateau *et al* 1940; Razet 1953, 1961; Desai and Kilby 1958b; Ross 1959; Lisa and Ludwig 1959).

Analysis of the cotton stainer *Dysdercus fasciatus* urine by Berridge (1965) for its nitrogenous end products revealed that unlike in other terrestrial insects, the main product in this insect was not uric acid but allantoin. The other components identified were urea, amino acids and a muco-polysaccharide which on hydrolysis yielded simple sugars and amino acids. Ultraviolet examination of the urine sample chromatogram, exposed a bluish green fluorescent spot which with ninhydrin gave a mauve coloured spot and with dimethyl-amino-benzaldehyde an orange coloured one. This compound was subsequently confirmed as kinurenine after comparing its properties with those of an authentic specimen of kinurenine. Likewise, Baker's (1976) analysis of the adult fecal extracts of the stored product insects *Sitophilus oryzae* and *Sitophilus granarius* revealed uric acid as the major purine base constituting more than 21% (w/w) of the faeces of the former and 17% of the latter. Other components identified, were small but significant amounts of hypoxanthine, xanthine and allantoin suggesting the existence of an active xanthine oxidase and uricase in both the insects. The presence of urea was also demonstrated. In an earlier experiment, Bhattacharya and Waldbauer (1972) identified hypoxanthine and uric acid as the major constituents in another stored product insect *Tribolium confusum* Jacquelin du Val maintained on different forms of wheat while Nation and Thomas (1965) recorded uric acid and xanthine as the major components in the excreta of *G. mellonella* fed on an artificial diet.

It is rather surprising that although the catabolic mechanism of uric acid the most important excretory product in insects has received much attention, its biosynthesis in them has curiously escaped it. Judging from its high concentration in insect excreta,

the possibility of the origin of uric acid exclusively from nucleic acids can be ruled out. This naturally leads one to assume protein nitrogen to be the source. Baldwin (1948, 1963) and Hoskins and Craig (1935) suggested the possible involvement of two amino acids—arginine and histidine as intermediaries in its formation but no pathway was confirmed. Among the earlier workers who studied the *in vivo* and *in vitro* formation of uric acid in insects, mention can be made of Leifert (1935), Brighenti and Colla (1940), Anderson and Patton (1955), McEnroe (1956) and Desai and Kilby (1958b). These authors, with a view to identify the precursors of uric acid in insects, tried several non-purine compounds (urea, ammonium salts, malonate, 4-amino-5-imidazole carboxamide and monoethyl oxaloacetate etc.) and observed them capable of stimulating uric acid synthesis in certain cases to some extent.

Subsequent work of Heller and Jezewska (1959) on *Antheraea* supported the above view when uric acid synthesis was stimulated by the simultaneous addition of precursors like formate, ribose-5-phosphate, glutamate and aspartate in the presence of ATP.

Ito and Mukaiyama (1964) confirmed the role of hypoxanthine and/or xanthine in the metabolism of protein nitrogen when feeding of increased quantities of proteins to *Bombyx mori* resulted in the significant increase of xanthine oxidase activity. Based on these observations and on the fact that adenase activity is overshadowed by that of guanase (Hayashi 1961a), a new pathway was suggested emphasizing an important involvement of guanine and xanthine. Even this however, has to be accepted with some reservation since it does not appear actually to be the case.

Earlier, McEnroe (1956) had demonstrated that injections of ammonia into the haemolymph of *Periplaneta* increased uric acid and urate deposition in the fat body. However, the possibility of the injected ammonia having given rise to various intermediates before the production of uric acid cannot be ruled out. In *Periplaneta*, the presence of the enzymes required for such biochemical transformation can be presumed based on McAllan and Chefurka's (1961a, b) demonstration of the occurrence of a very active dehydrogenase whose equilibrium is suggestive of the amination of α-ketoglutarate producing glutamate. In addition, the presence of glutamate-aspartate aminotrasferase also has been demonstrated catalysing the production of aspartate with the simultaneous disappearance of glutamate.

Theoretically there exist several possibilities for uric acid biosynthesis in insects and 3 generalized pathways have been considered to be involved in its production. They are, (i) the *de novo* synthetic process generally known as the uricotelic pathway where protein is the source of nitrogen, (ii) the uricolytic (Bursell 1967) or the degradative nucleicolytic pathway (Cochran 1975) where nucleic acids or their components are the starting material and (iii) the degradative pathway of uric acid in insect tissues, which should actually be known as the uricolytic pathway. The *de novo* synthesis pathway has been considered as the most important and convincing since it explains the formation of the large quantities of the compound produced in them. Although the proposal still leans much on the information available for aves, the existing evidence does support the fact that the pathway established for birds (Buchanan and Sonne 1946; Buchanan *et al* 1948; Buchanan 1951) and that followed by insects, have several similarities and might even be the same.

It has been well established that insects maintain a correlation between uric acid production and degradation of protein and amino acids (Terzian *et al* 1957; Tojo and Hirano 1968; Birt and Christian 1969; Mullins 1971; Tojo 1971). The earlier *in vivo* studies of McEnroe and Forgash (1957, 1958) on *Periplaneta* have shown that the

origin of carbon atoms 2 and 8 of the purine ring (figure 1) could be traced to the labelled formate and also that the C-6 of glucose and the C-3 of serine in *Drosophila* were traceable as primary sequential sources of the formate carbon. Similarly, labelled studies of Barret and Friend (1970) further substantiated the findings of Buchanan (1951) that all the 5 carbon atoms of the uric acid molecule have the same origin as that proposed for the aves. Thus the currently accepted and recognized *de novo* pathway for the synthesis of uric acid in insects is believed to start with 5-phospho-D-ribosyl-1-pyrophosphate. Substituents are added in the 1 position of the ribose molecule replacing the pyrophosphate moiety and this ultimately results in the building up of inosine-5-phosphate (IMP). Figure 1 represents the origin of carbon and nitrogen atoms for IMP production while figure 2 shows the schematic pathway of the *de novo* purine synthesis.

IMP having the potential to produce numerous compounds (Magasanik and Karibian 1960), formation of uric acid from it in a uricotelic organism readily occurs. Thus the uricotelic pathway involves dephosphorylation of IMP to inosine and removal of ribose by hydrolysis or phosphorolysis resulting in the formation of hypoxanthine which under the influence of xanthine oxidase gives rise to xanthine and ultimately produces uric acid (figure 3). No doubt, the pathway is a high energy demanding one since in the formation of one molecule of IMP about 8 high energy phosphate bonds are likely to be expended (Mahler and Cordes 1966).

Investigations on the localization of enzymes participating in purine metabolism in insects, have revealed that the fat body, midgut, hindgut or Malpighian tubules are their main sites. For instance, xanthine dehydrogenase has been observed to have higher concentration in the fat body of *P. americana* (Anderson and Patton 1954, 1955), in *Drosophila* (Ursprung and Hadorn 1961; Keller and Glassman 1963; Parzen and Fox 1964) and in a variety of other insects (Duchateau *et al* 1940; Florkin and Duchateau 1941; Keller *et al* 1963; Smith *et al* 1963). The presence of uricase has been detected in the fat body (Leifert 1935; Pierre 1964, 1965) as well as in the Malpighian tubules (Razet 1961; Nelson 1964). According to Razet (1961), in insects with active allantoinase activity, the enzyme distribution is the highest in the Malpighian tubules followed by the fat body and the midgut.
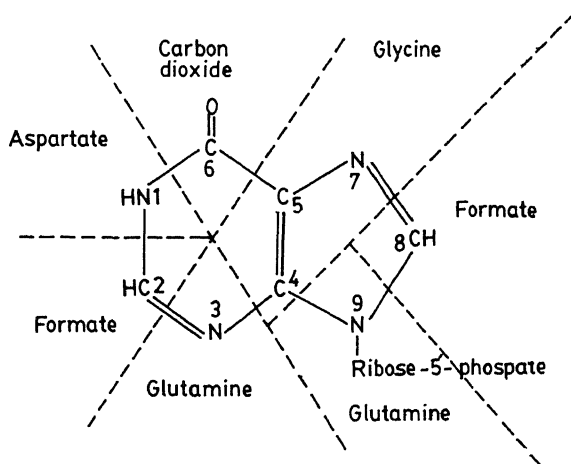
Figure 1. The origin of carbon and nitrogen atoms for inosine-5′-phosphate production in the *de novo* purine biosynthetic pathway (adapted from Bursell 1974 and Cochran 1975).
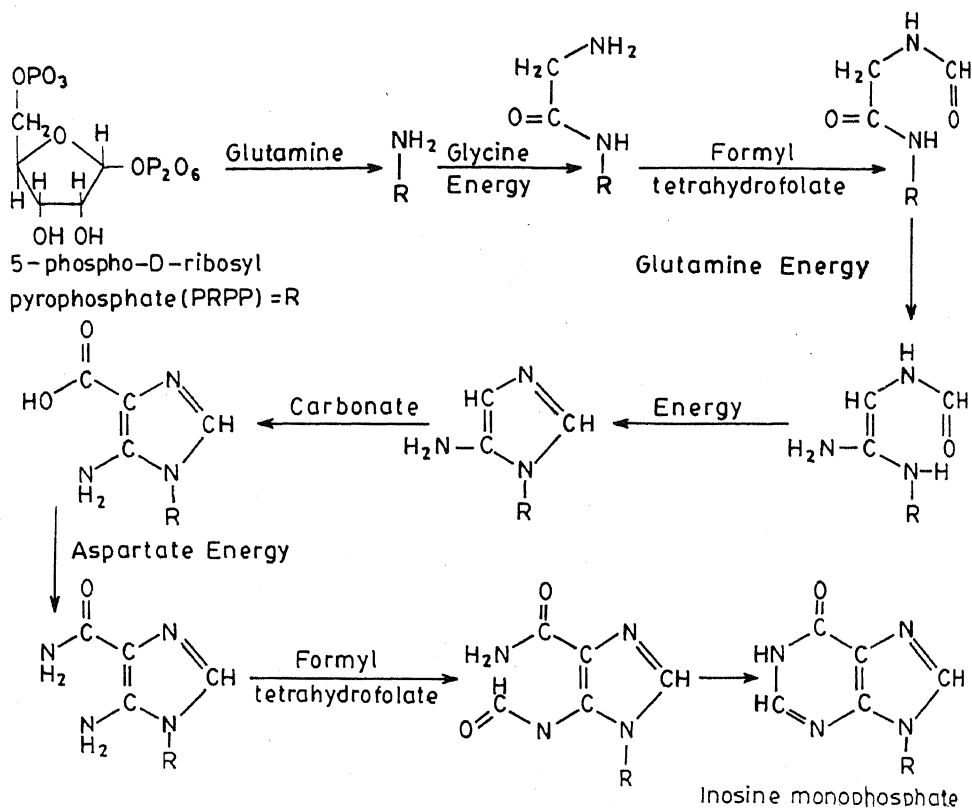
Figure 2. The *de novo* purine synthetic pathway (adapted from Cochran 1975).
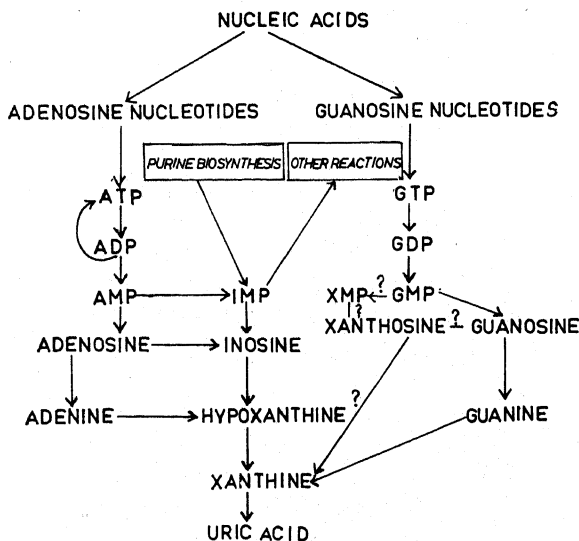
Figure 3. The nucleicolytic pathway for uric acid synthesis in insects (adapted from Cochran 1975).

Fat body has been established as the main tissue involved in uric acid synthesis (Cochran 1984). This conclusion was arrived at by tabulating all the available published data on the tissuewise distribution of adenase, guanase and xanthine dehydrogenase (the enzymes involved in the terminal steps of urate synthesis) when it was observed that the fat body especially at the larval stage of insects was the major source.

In vivo studies on insect fat body also evince its implication as the most important tissue for uric acid biosynthesis. Thus all recent studies lead to the conclusion that the *de novo* synthesis of uric acid in insects occurs in the fat body the mechanism as already stated being similar to that established for birds. However, it is noteworthy that evidence for the existence of a similar reaction sequence in them as recognized in the aves, is far from complete. In fact, only a few steps have unequivocally been established while nothing is known regarding the individual steps prior to the formation of IMP.

The second accepted mechanism for uric acid synthesis in insects is the nucleicolytic pathway which originates from the nucleic acids derived partly from the digested food materials and partly from the processes of tissue maintenance and repair (Bursell 1967). In contrast to the uricotelic pathway, the production of uric acid by the nucleicolytic pathway has been considered as rather small (Terzian *et al* 1957; Mullins 1971; Wigglesworth 1972).

It is also well known that nucleotides are involved in cellular energy conservation reactions wherefore their breakdown and destruction need be kept under control and their activities regulated (Johnson *et al* 1980a, b, c; Miller 1980). Thus the speculation that the nucleicolytic pathway plays only a minor role in the biosynthesis of uric acid stands further supported. Consequently, it will not be incorrect to assume that it is mainly via the *de novo* synthetic and to a small extent only by uricotelic pathways that the main bulk of uric acid production in insects takes place.

The nucleicolytic pathway also involves a large number of enzymes resulting in a number of reaction products. The reactions themselves have been considered to possess major functions, their excretory contribution being only incidental. The actual pathway, is represented in figure 3 and as it proceeds it follows the same reaction steps which the uricotelic pathway traces from IMP formation onwards.

Synthesis of nucleic acids in insects has been well established (Russo-Caia 1963; Forrest *et al* 1967; Moriuchi *et al* 1972; Chinzei and Tojo 1972; Miller and Collins 1973) and the presence of DNase demonstrated but their degradation studies in insects are scant. Nevertheless, the existing knowledge on the subject indicates that in insects an 'ebb and flow' of nucleic acid synthesis and degradation keeps on continuously taking place, the process depending on the age, stage of development, sex and the nutritional status of the insect and possibly on some other unknown factors as well. The conversion of nucleotides (products of nucleic acid degradation) into uric acid and the presence of several enzymes in various insect tissue cells capable of catalyzing nucleotide modifications—particularly those containing adenosine, are well established.

Many ATPases, some of them capable of dephosphorylating all the common nucleoside triphosphates, have been reported in a variety of insects (Sacktor 1953; Sacktor *et al* 1953; Sacktor and Cochran 1957; Maruyama 1954; McShan *et al* 1955; Avi-Dor and Gonda 1959; Mills and Cochran 1967; Berridge and Gupta 1968). Further, the inter-conversion of ATP, ADP and AMP has been established (Hofmanova *et al* 1967; Carney 1969) and isolation of specific ATPases in pure state

has also been achieved (Mills and Cochran 1966; Hofmanova *et al* 1967; Carney 1969). The earlier report of Gilmour and Calaby (1953) on the presence of myokinases in insects was confirmed when Mills and Cochran (1966, 1967) isolated pure myokinase from *P. americana*. Earlier, in *Musca domestica*, Sacktor and Cochran (1957) had demonstrated the removal of the second phosphate group from GTP. Thus from the existing knowledge available, it has been concluded that inter-conversion and/or degradation of adenosine and guanosine nucleotides to the monophosphate level in insects can easily occur.

Although the dephosphorylation of AMP, IMP and GMP has been reported with several enzyme preparations from *Periplaneta* (Cochran 1961; Cochran and Bruno 1963; Bruno and Cochran 1965), there appears to be insect species differences for the availability of the enzymes (Gilmour and Calaby 1952; Sacktor and Cochran 1957).

The 5'-nucleotidases produce adenosine, inosine and guanosine. While adenosine deaminase which converts adenosine to inosine has been identified in insects (Lennox 1941b; Wagner and Mitchell 1948; Desai and Kilby 1958b; Cochran 1961; Cochran and Bruno 1963; Cordero and Ludwig 1963; Bruno and Cochran 1965; Hodge and Glassman 1967a, b), guanosine deaminase, its counterpart remains unidentified (Duchateau *et al* 1940). The same holds good for xanthosine since this compound as well as AMP deaminase have not been reported in insects. The dephosphorylation of AMP has been considered by Cochran (1961) to occur prior to its deamination by *Periplaneta* muscle mitochondria but Hodge and Glassman (1967a) have claimed evidence for AMP deamination in *Drosophila*. Likewise, GMP deaminase also remains unreported and its product XMP has been considered as a rarely occurring compound in insect tissues (Cochran 1975).

Nucleosidases, have been claimed to accomplish the removal of ribose from nucleosides, the reaction being either hydrolytic or phosphorolytic (Cochran 1975). Inosine phosphorylase which catalyses the conversion of inosine' to hypoxanthine has been demonstrated in *Periplaneta* (Cochran and Bruno 1963; Bruno and Cochran 1965) and in *Drosophila* by Hodge and Glassman (1967a). Although the enzymes adenase and guanase which deaminate adenine to hypoxanthine and guanine to xanthine are well known (Duchateau *et al* 1940; Anderson and Patton 1955; Desai and Kilby 1958b; Lisa and Ludwig 1959; Prota 1961; Cline and Pearce 1963; Pierre 1965; Hopkins and Lofgren 1968), the conversion of adenosine to adenine and guanosine to guanine yet remains unclear.

Finally, the conversion of hypoxanthine to xanthine and xanthine to uric acid is catalysed by xanthine dehydrogenase (Irzykiewicz 1955). The presence of xanthine dehydrogenase has been well recognized (Hayashi 1961a, 1962) and demonstrated in the fat body as well as in the tissues of several insects (Riemke *et al* 1978). Figure 3 represents the reactions involved in the nucleicolytic pathway in the synthesis of uric acid.

The third pathway in insects involving uric acid is the uricolytic pathway where practically all steps are well established and recognized and those few that yet remain ambiguous have been attributed to either insect species difference or to their intracellular symbionts.

The first reaction in this pathway as shown in figure 4, is the conversion of uric acid to allantoin by the enzyme uricase. This complex reaction is considered to occur in two steps (Florkin and Duchateau 1943) with the formation of an unstable intermediate compound demonstrated in the wings of *Papilio* on injecting with $2\text{-}^{14}\text{C}$
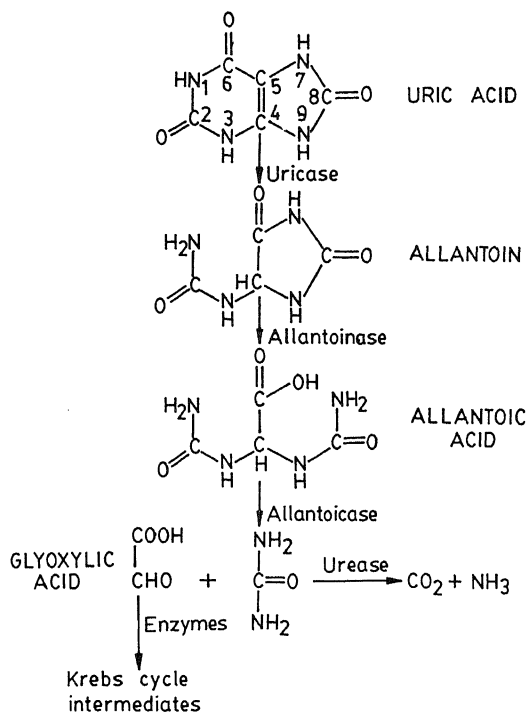
Figure 4. Uricolytic pathway in insects.

uric acid. This has been reported by Tojo and Yushima (1972) as an intermediate between uric acid and allantoin or a uric acid-allantoin conjugate.

The presence of uricase has been reported by several investigators in a variety of insect species (Leifert 1935; Robinson 1935; Truszkowski and Chazkinowna 1935; Rocco 1938; Razet 1952, 1953, 1954, 1956, 1957, 1961; Berridge 1965). The existing knowledge regarding tissue distribution of uricase is rather confusing and available data evince that the Malpighian tubules and perhaps fat body and the gut are the sites of uricase activity.

The conversion of allantoin formed in the first step to allantoic acid is influenced by the enzyme allantoinase. Although this enzyme has been detected in several insect species, its distribution in them is rather restricted (Razet 1961).

The third sequential reaction in the conversion of allantoic acid to urea and glyoxylic acid is catalyzed by the enzyme allantoicase. This enzyme has been detected perhaps in only two insects (Razet 1952, 1961, 1965; Wang and Patton 1969) and the information available is rather scant.

There are quite a number of insects which do not excrete all the degradation products formed in the uricolytic pathway and some even do not appear to reveal the presence of some of the enzyme participants. However, this in no way conclusively proves their total absence. It is quite likely that the concentrations of the undetected enzymes and metabolites are rather too low to be detectable by the methods employed. Notwithstanding this, it is also true that some insect species

exhibit the loss of uricolytic activity during certain stages of their development. For instance, in *Popillia japonica*, the embryonic and larval extracts exhibit uricase activity but those of the pre-pupa and pupa do not (Ross 1959). Likewise, the blowfly pupa *Lucilia* loses its uricolytic activity during development (Brown 1938a).

Results of McCabe (1973) on tsetse flies suggest that uric acid synthesis in this insect follows the normal pathways with carbon dioxide, formate and glycine as the main carbon precursors. Although the amount of glycine and that of its main precursor serine formed during digestion is inadequate to account for the amount of uric acid formed, it is likely that the supply of carbon is supplemented by conversion of alanine to glycine via serine (Bursell *et al* 1974). Albeit, the existence of a totally different pathway for uric acid synthesis in insects, is quite possible.

Urea has been found to be absent or present in minute quantities in excreta of insects even in the presence of allantoicase activity. In cases of its total absence in insect excreta it can be correlated with the apparent scarcity of allantoicase. Under such circumstances, the breakdown beyond the stage of allantoic acid could be considered as not of much significance in insect excretory metabolism unlike in that of mammals.

The last enzyme participating in the uricolytic pathway is urease which catalyses the hydrolysis of urea to carbon dioxide and ammonia. However, the presence of urease in insects in general, is still controversial and an open question despite its reported presence in some carnivorous dipteran insects and in a few others (Baker 1939; Robinson and Baker 1939; Robinson and Wilson 1939; Razet 1961). In some insects where the uricotelic pathway is not operative and the source of nitrogen happens to be proteins, the amino nitrogen is excreted as ammonia while in some others insignificant quantities of urea are excreted. In other species, specific amino acids like arginine, histidine and cystine are excreted in the free form.

The nitrogenous end products of insects have different nitrogen percentage content and different solubility in water. For instance, ammonia and urea the most soluble excretory products in insects contain 82 and 46% of nitrogen respectively and both being highly soluble in water, are ideal media for removal of waste nitrogen. The purine bases adenine, guanine, hypoxanthine, xanthine and allantoin etc., on the other hand, contain between 30–40% of nitrogen and are much less water soluble than both urea and ammonia while arginine and histidine barring cystine, although not much different in nitrogen content from the purines, are appreciably soluble.

2. Pyrimidines

In addition to the purine bases, nucleic acid has another type of components, viz., the pyrimidines. Unfortunately however, much information concerning their metabolism in insects is not available despite the fact that they could be utilized in the synthesis of additional nucleic acids. This is substantiated by detection of several enzymes like cytidine deaminase, and uridine phosphorylase which can act upon pyrimidine nucleotides (Bruno and Cochran 1965).

According to some studies, the metabolic pattern of pyrimidines is believed to be similar to that in the vertebrates (Bruno and Cochran 1965). Nevertheless, much more work yet remains to be carried out before assigning them specific roles as nitrogenous end products.

Urea

Another very important nitrogenous end product the biosynthesis whereof in insects is and has ever been a controversial topic needing special mention is urea. The presence of urea as a minor constituent of insect haemolymph (Buck 1953), tissues (Leifert 1935) and of excreta (Razet 1966; Bursell 1967; Corrigan 1970; Schoffsniels and Gilles 1970; Wigglesworth 1972; Lazar and Mahamed 1979) is well known. Some insects have also been reported to void larger amounts of urea (Powning 1953; Berridge 1965) but irrespective of the quantities excreted, urea has been considered of metabolic origin rather than from the dietary source (Gilmour 1965).

Ureotelic animals can synthesize urea by adopting two pathways viz., either via degradation of purines through uric acid (uricolytic pathway) or by the classical ornithine cycle. However, production of urea by the former pathway is out of the question since the uricolytic pathway does not function beyond the formation of allantoic acid and in the latter pathway, the ornithine cycle has been considered non-functional in insects (Cochran 1975). According to this, the insect tissues form a part of the vertebrate ornithine cycle, the most important among them being the enzyme arginase which catalyzes arginine to ornithine and urea. The presence of arginine has been demonstrated in insect fat body and muscle (Reddy and Campbell 1969a, b; Tsuyama *et al* 1980) while some or all the enzyme participants in ornithine cycle have been detected by others (Powles *et al* 1972; Pant and Kumar 1978).

The detection of significant quantities of arginase practically in all insects and of arginine, citrulline and ornithine in many, has been made at all stages of development (Garcia *et al* 1956a, b; Kilby and Neville 1957; Szarkowska and Porembska 1959; Hayashi, 1961b; Porembska and Mochnacka 1964; Reddy and Campbell 1969a). However, the nutritional and enzymic studies hitherto made on them (House 1965; Porembska and Mochnacka 1964; Reddy and Campbell 1967; Inokuchi *et al* 1969), have not given any conclusive and unequivocal evidence regarding the operation of a Krebs-Henseleit urea cycle, a topic which still remains under dispute and highly controversial.

Powles *et al* (1972) studied the urea cycle enzymes in the vegetable bug *Nezara viridula* and observed that of the 5 participant enzymes in the ornithine cycle, arginase and ornithine transcarbamoylase were active but there was no evidence for the arginine synthetase system. In addition, carbamoyl phosphate synthetase exhibited very low activity and was detectable only by radioactive substrates. Based on these it was concluded that urea in *N. viridula* was formed exclusively from excess of dietary arginine via the action of arginase.

The report of Pant and Kumar (1978) on the presence of appreciable activity of the urea cycle enzymes in the tissues of the dipteran carnivorous *Sarcophaga ruficornis* (flesh fly) all through its life-cycle appears significant in contradistinction of their total absence except arginase (L-arginine ureohydrolase), (EC 3·5·3·1) in the phytophagous lepidopteran eri silkworm *P. ricini* at any stage of development. Therefore, the presence and significant variation of all these enzymes, all through development of *S. ruficornis* suggests the possibility of the operation of the Krebs-Henseleit urea cycle in this carnivorous insect. However, confirmation of these findings *in vivo* by employing [^{14}C]-ornithine need be made when conclusive and unequivocal evidence regarding the operation of a urea cycle in an insect can be obtained.

4. Ammonia

Existence of a correlation between the excreted nitrogenous material by living organisms and their natural environment was first formulated by Needham (1938). Thus excretion of ammonia by aquatic animals is a common feature whereas their terrestrial cousins excrete a variety of products and require less water elimination. In fact, it was perhaps Weinland (1906) who first demonstrated that insect larvae that lived in water or moist environment are strongly ammonotelic. Subsequently, several investigators (Staddon 1955, 1959; Gilmour 1961; Wigglesworth 1972) confirmed ammonia as a major excretory product of several aquatic species of insects.

Mullins and Cochran (1972) demonstrated ammonia to be the principal nitrogenous excretory product in *P. americana*—an insect considered totally uricotelic—under certain dietary conditions. In addition, numerous species of blowfly larvae are also known to void large amounts of ammonia in their excretion (Lennox 1940, 1941a; Cochran 1984).

As with several other nitrogenous excretory products, the exact mode of ammonia formation, its precise role in insects and the form in which it is excreted are yet to be clearly understood. Nevertheless, it has been well established that it is a cation in the excretion of several insects and particularly a major one in *Periplaneta* influencing its water balance (Mullins 1974).

Under normal conditions *P. americana* excretes ammonia despite significant internal storage of nitrogen as uric acid (Mullins 1974) and also releases it in small quantities during dietary nitrogen starvation only when stored reserves of uric acid are available (Mullins and Cochran 1975a, b).

In insects where only insignificant quantities of ammonia are excreted its formation could be attributed to the deamination of purines, the breakdown products of nucleic acids. However, where they occur in large quantities proteins appear to be the source and there are several ways by which protein nitrogen can give rise to ammonia, one of them being the deamination of amino acids (Bheemeswar 1959). In fact, very active amino acid oxidase activity has been demonstrated in the fat body (Kilby and Neville 1957; Desai and Kilby 1958a) and Malpighian tubules of several insects (Auclair 1959; Corrigan *et al* 1963; Boadle and Blaschko 1968). Curiously though, experiments with amino acid-fed insects neither resulted in increased quantities of ammonia excretion nor their larval homogenates were able to deaminate amino acids. However, when proteins were fed to them, increased ammonia excretion occurred and what is more, even larval homogenates evolved ammonia from peptone mixtures (Brown and Farber 1936; Brown 1938b) leading to the conclusion that the excreted ammonia was liberated by deamination of proteins or their breakdown products.

Another mode of ammonia production in insect excreta could be via transamination reaction of amino acids with α-ketoglutaric acid when the glutamic acid formed may be deaminated by glutamic dehydrogenase, which has been detected in a variety of insects (McAllan and Chefurka 1961a, b; Zandee *et al* 1958; Murphy and Micks 1964; Chen and Bachmann-Diem 1964; Emmerich *et al* 1965).

Examination of fecal pellets by Mullins (1974) collected from *P. americana*, maintained on 12 different diets containing various levels and sources of nitrogen revealed that ammonium ions were the major cations excreted in increasing amounts with the increase in dietary nitrogen level. This led to the speculation that the mode of ammonia elimination was associated with the gut and not from respiratory

surfaces in spite of the uncertainty regarding the genesis of gut ammonia due to its involvement with the gut antibiotic resistant microflora (Todd 1944). It is quite possible that ammonia does get released via gut tissue.

The *ad libitum* water requirements of *Periplaneta* have been correlated by Mullins (1974) with dietary nitrogen levels and ammonia excretion. The presence of high concentrations of cations and especially of ammonium ions in the excretion has been considered as responsible for the increased water requirements in cockroaches which facilitates the expulsion of a moist fecal pellet. Hind gut is believed to participate in ammonia excretion in *Periplaneta* (Prusch 1971; Mullins 1974) and in blowflies (Lennox 1941a). However, in *Sarcophaga*, Prusch (1972) demonstrated both the fore and the hind guts as major sites of ammonia accumulation in varying concentrations.

A thorough examination of nitrogen excretion in different ammonotelic organisms has led to some very interesting and important revelations. For instance, Wieser *et al* (1969), Wieser and Schweizer (1970) and Wieser (1972a, b) reported that although most of the ammonia produced by the terrestrial isopods is excreted via its dissolution in the urine, a portion is also perhaps eliminated in the gaseous form—an observation earlier demonstrated by Speeg and Campbell (1968) in the land snails. In contradistinction, in earthworms (McDonnell and Tillinghast 1973), blowflies (Prusch 1972) and aquatic neuropterans (Staddon 1955) ammonia was demonstrated mainly associated with gut tissues and excreted with fecal materials. Dietary nitrogen has been shown directly related to excreted nitrogen in *Lumbricus terrestris* (Tillinghast and Janson 1971) and in *Columbia livia* (McNabb *et al* 1972) to the nitrogen storage as well, as urates (Haydak 1953; McEnroe 1956; Mullins and Cochran 1974, 1975a, b).

Use of different dietary nitrogen sources and at different levels (labelled uric acid and ammonium chloride) in the study of ammonotelic response, led to the detection of labelled uric acid in the fat body of *P. americana* (Mullins and Cochran 1972) while earlier Sedee (1958) had noted the utilization of dietary ammonia for amino acid synthesis.

Prusch (1972) has suggested that although ammonia excretion in the blowfly functions primarily in ridding of waste nitrogen, it is perhaps also concerned with the conservation of sodium and potassium ions.

There are several other reactions which can give rise to ammonia formation in insects from protein nitrogen. However, the evidence available, does not permit one to assess the contribution made individually by these different reactions towards the total output of ammonia. As a result notwithstanding all the information available to date on ammonia excretion in insects in general, the exact mechanism of ammonia formation or its precise substrate wherefrom it originates still remains unclear.

5. Amino acids

One of the salient features of insect biochemistry is the occurrence of high concentration of free amino acids in insect haemolymph and tissues which is often about 60 times higher than in the human plasma. This was probably first observed by Nazari (1902). This marked ability of insects to accumulate them induced Florkin (1949) to term the phenomenon as a systematic biochemical characteristic of insects. As such excretion of amino acids in varying quantities by several insect species (Razet 1966; Bursell 1967, 1970; Wigglesworth 1972; Pant *et al* 1984) should not at all be surprising. In fact, the presence of all amino acids participating in protein

synthesis as well as of some unusual ones like the α-amino-butyric acid, homoarginine, β-alanine and some guanidine bases, other than arginine has been detected both in haemolymph and insect excreta (Pant and Agrawal 1963).

Proteins being the main constituent of insect food, get easily digested by the insect gut. The amino acids of dietary origin pass out unabsorbed along with other amino acids produced in the gut by interconversion (Schoffniels and Gilles 1970) or/and with those contributed by the micro-organisms—may be, not as a true excretory product but as a gut function.

Insects eliminate amino acids in 3 different ways in addition to the one via gut. Some are excreted in small quantities, some in excessive amounts of those derived from diet while some specific ones get excreted in large quantities (Cochran 1984).

The normal excretion of amino acids in small quantities occurs through Malpighian tubules as well as via rectum where reabsorption of some specific amino acids occurs (Wall and Oschman 1970; Balshin and Phillips 1971). These findings evince the existence of a normal excretory system of amino acids despite there being a possibility of their unavoidable loss due to their incomplete reabsorption by the rectum. The excretory origin of amino acids receives further support from the report that the boll weevil excreted 20 different amino acids constituting about 3% of the total nitrogen voided while its diet was totally devoid of any α-amino nitrogen (Mitlin et al 1964). Similarly, honey bees maintained on protein-free diet, excreted amino acids amounting to about 1% of total nitrogen eliminated (McNally et al 1965).

Excretion studies on α-amino nitrogen ingested in excess of dietary requirement, have mainly been carried out on plant sucking insects. Several insect species, especially aphids live on plant saps which are rich in protein, amino acids, amides and several other organic materials and far exceed particularly in α-amino nitrogen content required normally for insect nutrition. The excreted material by these insects known as honeydew is equally rich in amino acids and other constituents as the plant sap but different in composition. In some cases, the plant sap and the honeydew both have the same amino acid composition qualitatively but differ quantitatively while in others the insect haemolymph also has the same composition as the sap and the honeydew but the amount of amino acids excreted is much less than that present in the sap. Amino acids initially not identified in the sap, have sometimes been detected in the honeydew while in some cases the number of amino acids recorded in the honeydew is far more in number than detected in the sap. For instance, the honeydew of *Kerria* registered 17 amino acids as against only 9 detected in the host plant (Srivastava and Varshney 1966). Likewise, of the 27 amino acids identified in *Nezara* (Powles et al 1972), only 19 were initially detected in the plant sap. These variations in the amino acid composition of the dietary plant sap origin and the excreted honeydew have been attributed to the difference in sap origin, seasonal changes, species difference of insects, their nutritional status and ability to modify and effect amino acid interconversion and may be to some other factor(s) yet to be explored.

Thus it appears that honeydew production in plant-sucking insects serves a multipurpose means whereby they can imbibe at will amino acids and other sap components, retain them selectively, modify and interconvert them to suit their requirements and ultimately get rid of the excessive and unwanted ones along with water in order to maintain an internal osmotic balance and a regulated internal environment.

Riddance of high levels of specific amino acids via excretion has been observed in some insects. The classic chemical analysis of the excretory products of *Glossina morsitans* by Bursell (1965a, b) can be quoted as an example. The most striking feature of the excretory metabolism of this insect was the elimination of large amounts of arginine and histidine parallel to that of uric acid. Together, they accounted for about 20% of the total nitrogen voided which is rather high as compared with most other insects (Wigglesworth 1950; Prosser 1952; Craig 1960). In addition, small quantities of other amino acids besides arginine and histidine, substantial amounts of haematin and traces of ammonia and urea were also detected in the excreta. This finding supported by that of Moloo (1977) confirmed the complete elimination of these two nitrogen-rich amino acids arginine and histidine, as true excretory products in *G. morsitans*. It also evinces the operation of a specific mechanism whereby the elimination is accomplished. It is quite likely that certain conditions prevailing in the insect rectum induce precipitation of these amino acids as in the case of uric acid in *P. americana* (McNabb and McNabb 1980). Similarly, in *Bombyx* larvae Kondo (1967) and Levenbook *et al* (1971) in *Manduca sexta* meconium recorded histidine as a major excretory product. In the latter, ornithine was also detected in appreciable quantities. Another instance where a similar specific mechanism could be operative for riddance of amino acids is that of the clothes moth *Tineola biselliola* which excretes a substantial amount of cystine (Waterhouse 1952; Powning 1953) traceable to its wool diet consisting of keratins—the proteins rich in sulphur containing amino acids.

There is a possibility that the above cited excretion of amino acids is a loss to the insects rather than a riddance of waste amino nitrogen. However, in cases where considerable quantities of specific amino acids are voided (Powning 1953; Bursell 1965a, b) it indicates the riddance of waste nitrogenous products. Appearance of hippuric acid in *Bombyx* excreta (Shyamala 1964) has been interpreted as a detoxification mechanism for riddance of excess glycine.

Chemical analysis of the meconial fluid of two lepidopteran insects *P. ricini* and *A. mylitta* (Pant *et al* 1984) revealed high concentrations of α-amino nitrogen. Practically all amino acids identified in protein synthesis were detected with the predominance of arginine and lysine.

In *Neodeprion sertifer*, the α-amino nitrogen excreted amounting to 1–2% mainly constituted significant quantities of proline, hydroxyproline and histidine in comparison to other amino acids excreted (Fogal and Kwain 1974). In all probability, they represent the excessively ingested amino acids voided as true excretory products synthesized by the insect to rid of unwanted nitrogen.

On account of their exceptionally high titre in insect haemolymph and tissues, amino acids have not been considered as important excretory products. Maddrell (1981) attributed this partly to the slow functioning of the system which reduces their loss via Malpighian tubules where existence of membrane permeability barriers is speculated which further reduce their loss (Maddrell and Gardiner 1980). The reabsorption of amino acids in the rectum further facilitates their recovery and retention in high concentration in the amino acid pools of haemolymph and tissues.

6. Tryptophan and tryptophan derivatives

Insects are known to have an active tryptophan pathway (Gilmour 1961; Corrigan 1970) involving kinurenine and 3-hydroxy-kinurenine leading to the synthesis of

ommochromes the insect pigments (Butenandt 1952; Kikkawa 1953; Linzen 1974). These compounds along with ommins constitute a class of compounds which abundantly occur as pigments in several insect tissues especially in eyes, cuticle, wings, gonads, eggs, the Malpighian tubules and several other internal organs (Butenandt 1959; Butenandt *et al* 1960; Wessing and Bonse 1966). Brunet (1965) and Buckmann *et al* (1966) considered the production of ommochromes as a means of eliminating excess of tryptophan. This concept gains support from Linzen's (1974) observation regarding the tryptophan titre in insect tissues as a matter of concern and the subsequent find by Kayser (1979) of tryptophan toxicity in insects.

Ommochromes were the first tryptophan derivatives to be reported as insect excretory products (Goodwin and Srisukh 1950). Since then several investigators confirmed their occurrence in many other insect species (Butenandt *et al* 1960; Harmsen 1966a; Ogawa and Hasegawa 1980; Stratakis and Egelhaaf 1980). In addition to ommochromes, kinurenine is another tryptophan derivative often detected in lepidopteran excretion and their meconium fluids generally accompanied by traces of 3-hydroxy-kinurenine (Umebachi and Yamada 1964; Umebachi and Katayama 1966; Kayser 1979). In *Pieris* larvae Kayser (1979) has reported the excretion of kinurenine in quantities directly proportional to the dietary tryptophan content. The same was observed in the urine of the hemipteran *Dysdercus* (Berridge 1965).

Mullins and Cochran (1973) while examining the excretory products of *P. americana* noted 3 fluorescent substances which were identified as metabolites of tryptophan viz., kinurenic, xanthurenic and 8-hydroxy-quinaldic acids the concentrations whereof increased with increased intake of nitrogen by the insects. Quantitation of these compounds accounted for 1–3% of the total excretory nitrogen. Although Mullins and Cochran (1973) were the first to detect 8-hydroxy-quinaldic acid in *P. americana*, the isolation of the methyl ester of this compound was earlier effected by Schildknet *et al* (1969, 1971) from the prothoracic defence scent glands of the beetle *Ilybins fenestratus*.

In addition to its role as a participant in protein synthesis tryptophan has been considered to be involved in 4 other biochemical reactions which have been elaborated and well reviewed by Cochran (1975). However, in insects, the reactions occurring are limited to only two pathways viz., the kinurenine 3-hydroxy-kinurenine ommochrome and the 5-hydroxy-tryptophan-5-hydroxy-tryptamine (serotonin) pathways that are considered of importance.

Although serotonin has been recognized as an active compound in insect nervous tissues (Colhoun 1963; Maddrell *et al* 1971; Prince and Berridge 1973; Evans 1980), the concentration of this compound detected in insects is extremely low for its being considered as a participant in nitrogen excretion and hence it is the tryptophan-ommochrome pathway which operates in insects. Irrespective of the concentrations and quantitative appearance in insect excretion of the intermediate compounds in this pathway, it is the number of compounds appearing that is surprising. The important pathway of tryptophan metabolism in insects as reproduced from Cochran (1984) is represented in figure 5. The intermediate compounds that get formed in this pathway are either excreted by insects directly, accumulated internally and used by them as pigments deposited in their wing scales or converted to other compounds and accumulated or excreted in small quantities or even in traces.

Thus one can see that several tryptophan derivatives and intermediates that are formed in the ommochrome synthesis pathway have been detected in many insect
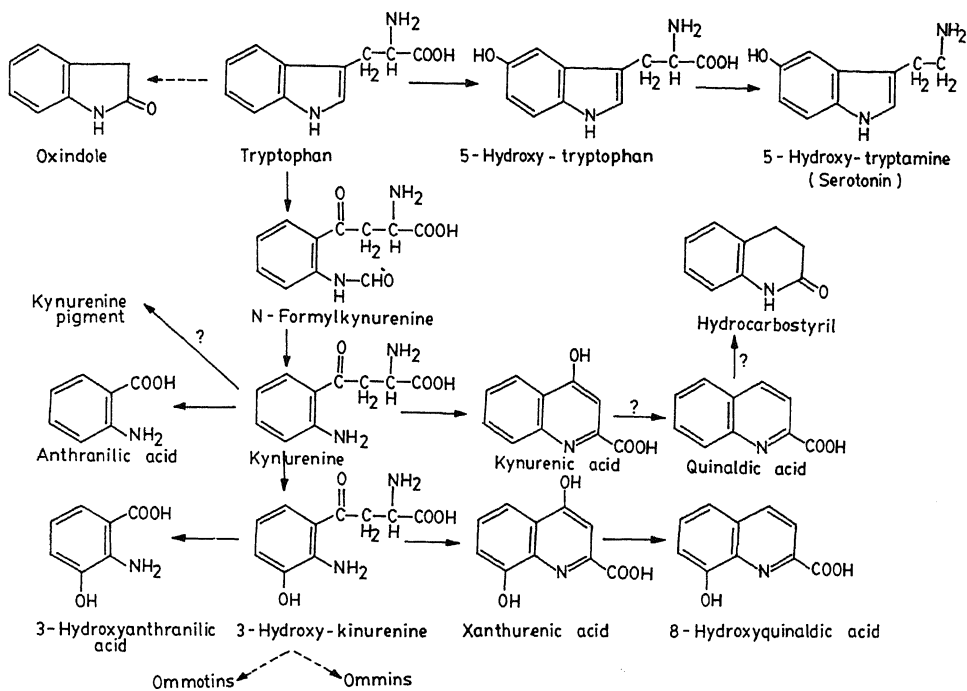
Figure 5. The tryptophan-ommochrome pathway in insects (adapted from Cochran 1975, 1984). Question marks denote unestablished reactions in insects and dotted arrowheads involvements of several reactions.

species excreta. Some of the important ones identified are kinurenic acid (Leibenguth 1967; Mullins and Cochran 1973; Berthold and Buckmann 1975; Stratakis 1979, 1980), xanthurenic acid (Leibenguth 1967; Mullins and Cochran 1973), oxindole and hydrocarbostyril (Block and McChesney 1974) and 8-hydroxy-quinaldic acid (Mullins and Cochran 1973). This compound was traced to the gut microbes since feeding of antibiotic resulted in the suppression of its excretion without affecting the other components viz., kinurenic and xanthurenic acids. The suppression was presumably the result of inhibition of gut microbial dehydroxylation of xanthurenic acid. The other intermediates detected were, 4,8-dihydroxyquinoline (Inagami 1955), 3-hydroxy-kinurenine glucoside (Linzen and Ishiguro 1966) and L-dihydroxy-xanthurenic acid (Brown 1965). As a matter of fact, even tryptophan has been detected as an excretory product in the blowfly *Protophormia terraenovae* during certain stages of its development (Linzen and Schartau 1974).

Although the exact excretion mechanism of tryptophan and its derivatives is not clear, it has been assumed that the insect haemolymph transports these substances to the Malpighian tubules in the dissolved state wherefrom they are carried into the tubule lumen and excreted. This mode of excretion well explains the elimination of even small quantities of substances. In *Drosophila* larvae however, Sullivan *et al* (1980) have suggested a tryptophan transport system in the Malpighian tubules. Detection of some enzymes by Berthold (1976) and Ogawa and Hasegawa (1980) in the Malpighian tubules capable of converting tryptophan into one or more intermediates during the process of excretion, gave some support to this concept.

However, the absence of all pertinent enzymes proves the improbability of the existence of this transport system.

The insect fat body has been considered to play a central role in the tryptophan-ommochrome pathway since all the enzymes actually concerned in this pathway have been located in this tissue (Cochran 1975). Thus it can be presumed that the fat body controls the production of all tryptophan derivatives, the haemolymph transports them and the Malpighian tubule-rectum complex voids them.

Maintaining *P. americana* on high protein or high tryptophan containing diets induced high mortality (Mullins and Cochran 1973; Bignell and Mullins 1977). This was attributed to the quinoline derivatives which act as active mutagens or carcinogens in mice (Bryan *et al* 1964; Kuznezova 1969). This led these authors to believe xanthurenic and 8-hydroxy-quinaldic acids as the cause of gut tumour formation in *P. americana* resulting in high mortality. However, it is rather surprising that Nature should have evolved in insects such a system for producing pigments which while lending beauty and elegance to them should also prove a constant threat to their very existence! May be, that this danger has been very efficiently and effectively avoided by insects via maintaining the tryptophan-ommochrome pathway under strict control.

7.   Pteridines

The first investigation on the brilliant fluorescent multicoloured wing pigments occurring in some lipidopteran insects, dates back to the year 1895 when Hopkins identified them as uric acid or/and urates. Wieland and Schopf (1925) named them as pterines but subsequently they were re-named as pteridines (Schopf and Becker 1936). Following the structural elucidation of leucopterin by Purrmann (1940) and Schopf and Reichert (1941), chemical studies on this group of compounds gathered momentum and substantial knowledge regarding these compounds accumulated (Pfteiderer and Taylor 1964).

Occurrence of pteridines in insect excreta amounting to 5% of the total nitrogen excreted (Bartel *et al* 1958; Hudson *et al* 1959) was followed by extensive research by several investigators (Butenandt 1959; Ziegler 1961; Gilmour 1961, 1965; Chefurka 1965; Bursell 1967; Ziegler and Harmsen 1969; Corrigan 1970; Chen 1971; Wigglesworth 1972) to study their role in insect biochemistry. Unfortunately however, beyond their chief role in insect pigmentation a property similar to that observed in the ommochromes the tryptophan breakdown products, the knowledge regarding their exact role in insect excretion remains limited and yet to be established.

Pteridines and ommochromes have quite a few points of similarity. Both groups of compounds are minor excretory products whose role in insects has not been clearly understood. Both get internally deposited (storage excretion) and pigmentation has been presumed as their primary role in insects where they often occur together in various tissues (Gilmour 1965).

Pteridine granules have been located in *Bombyx* larvae cuticular epithelial cells (Sakurai and Tsujita 1976a), the genetic mutants exhibiting granular variations (Sakurai and Tsujita 1976b). The externally excreted amounts of pteridines are rather small but significantly large quantities are deposited internally. In *Pieris brassicae* Lafont and Pennetier (1975) observed significant quantities of pteridines stored in the

general body cuticle and wing scales. Likewise, Harmsen (1966a,b,c) recorded increasing concentrations of pteridines during pupal development of this insect (*Pieris brassicae*) followed by the voiding of about 2% of the total pteridine content with the meconium (Harmsen 1966a, c).

The biologically important pteridines are mostly the derivatives of 2-amino-4-hydroxy pteridine where substitutions take place at the 6 and/or 7 positions as represented in structure 1. This group of compounds are generally known as pterines.

Insect fecal material has been found to contain some pterines. For example in *Oncopeltus*, the presence of xanthopterin and isoxanthopterin has been detected while the wild type *Drosophila* meconium records the presence of isoxanthopterin and 2-amino-4-hydroxy-pteridine (Kursteiner 1961) and the Malpighian tubules exhibit the presence of tetrahydro-biopterin, xanthopterin, drosopterin, neodrosopterin and riboflavin (Wessing and Eichelberg 1968).

Some of the biologically important pteridines that are detected in insects in varying quantities are, biopterin, xanthopterin, isoxanthopterin, leucopterin, sepiapterin, isosepiapterin, erythropterin, 7-methyl-xanthopterin, violapterin, chrysopterin, riboflavin and a few unidentified ones. These are generally located as pigments in the insect compound eyes, wing scales, integuments, eggs and some internal organs.

Pteridines are related to riboflavin—one of the important vitamins of the B-complex group and the cofactor folic acid which along with methionine the sulphur containing amino acid, is associated with hepatic cirrhosis treatment. The role of pteridines as cofactors in the conversion of phenylalanine to tyrosine (Kaufman 1967) is well known. Folic acid is a conjugated pterine which insects are incapable of synthesizing and hence its supply has to be through dietary source. Riboflavin, a dietary requirement of insects (Friend 1958) is a derivative of 2, 4-dihydroxy pteridine or lumazine and has been detected in Malpighian tubules (Busnel and Drilhon 1942; Wessing and Eichelberg 1968). However, synthesis of riboflavin in insects is uncertain. Due to the biological importance of these compounds, modern pteridine research is mostly focussed on the various reactions involved in the inter-conversion of these compounds and the various enzyme participants catalyzing these reactions (Gyure 1974; Fan *et al* 1975; Dorsett *et al* 1979; Krivi and Brown 1979; Mazda *et al* 1980).

Excretion of pteridines, although traceable to dietary source in some insect species (Ziegler and Harmsen 1969) the compounds could also be of endogenous origin. The continuous substantial increase in pteridine concentration observed during the pupal development of *Pieris brassicae* (Harmsen 1966a, b, c) is in support of this concept. Further, the removal of one of the insect wings (a storage site) increased excretion and fat body storage of these compounds in the insect only to a small extent but
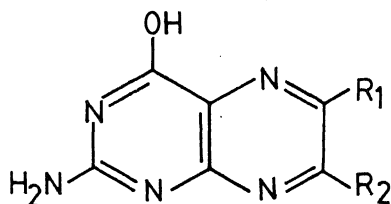
Structure 1. Pterine formation.

significantly reduced the total synthesis of pterines (Cochran 1975). This induced Harmsen (1966a) to consider pterines as important metabolic products which functioned as pigments in insects and traceable to *in vivo* synthesis. Judging from their adaptive nature of pigmentation in insects as recorded by Brower (1969) and supported by Corrigan (1970), Nolfi (1970) assigned to these compounds pigmentation as the primary role in insects. Thus pterines and pteridines play a dual role viz., that of pigmentation and involvement in the process of excretion.

Pteridines play a special role in insect eyes which are compound in nature and light perception does not entirely depend on either pteridines or ommochromes (Goldsmith and Warner 1964). These pigments are located in the eye pigment cells and distributed in a specific manner (Ziegler 1961) their function being to screen the pigments as light filtering substances (Langer and Hoffmann 1966). They also function in contrast perception (Wehner *et al* 1969).

An examination of pterine content organwise by Egelwaaf (1956) in 3 genotypes of *Ephestia* revealed that all tissues recorded the presence of isoxanthopterin and all except midgut had xanthopterin, 2-amino-6-hydroxypterine and an unidentified pterine. The highest concentration was located in the eyes followed by the Malpighian tubules, testes and ovary. All pterines formed the integrated parts of the cell and their distribution susceptible to changes during development.

Fat body has been considered as the site of pteridine synthesis. This was demonstrated by Harmsen (1966c) in *Pieris*. He studied the distribution of leucopterin and isoxanthopterin during pupal and pharate-adult development of this insect and noted the accumulation of both the pterines in the wings of the insect just prior to adult emergence. Preceding this, both the compounds increased significantly and subsequently declined precipitously. Leucopterin was observed to increase in particular with pupal development. Concurrently, the fat body got depleted of its pteridine content especially in leucopterin content. This conclusively proved the fat body as the site of synthesis of xanthopterin and leucopterin type of pteridines and the haemolymph playing the role of transporting them to their sites of excretion or storage.

Pterines were initially considered as excretory substances which also functioned as ornamentation in insects (Hopkins 1895). This observation along with Becker's find (1937) that pterines are unexcretable due to their inability to pass the Malpighian tubule barrier, gave rise to the concept of storage excretion of pteridines in which nitrogen containing highly oxidized molecules were immobilized in insect wing scales (Harmsen 1966a).

Thus from the data available and from his own work, Harmsen (1966a) speculated the existence of a strong but undefined excretion barrier to the voiding of pteridines especially during meconium formation at the Malpighian tubule level. This favoured the retention of pterines and resulted in their accumulation in insects. Albeit, exceptions to the excretion barrier concept have also been demonstrated in *Mylothsis* which does not exhibit a barrier for 2-amino-4-hydroxy pteridine—a pterine (Harmsen 1969), and in *Phonoctonus* which ingests large quantities of pteridines and probably voids them through faeces rather than via Malpighian tubules (Ziegler and Harmsen 1969).

The biochemical origin of pteridines (Brown 1971), their chemistry, metabolism and involvement in insect pigmentation and excretion have been extensively worked out and elaborately documented by Ziegler and Harmsen (1969), Corrigan (1970) and Cochran (1975).

The genesis of pteridines has been well established as occurring from a purine precursor-probably a guanosine nucleotide (Fan *et al* 1976) and the enzyme GTP cyclohydrolase as the enzyme that catalyzes the initial steps in the building up of the pteridine molecule. Watt (1967) proposed the formation of an initial pteridine from a guanosine precursor whereas Rembold and Gyure (1972) forwarded alternate pathways for the pterine ring synthesis. However, it is now well recognized that it is the purine molecule which is the precursor of pteridine in insects (Cochran 1984).

Figure 6 represents the pathway of purine conversion to pteridine and figure 7 the pterine biosynthetic pathway occurring in insects, as reproduced from Cochran (1975).

In *Oncopeltus fasciatus* eggs Smith and Forrest (1976a, b) detected the presence of a specific isoxanthopterin binding protein. This protein has been speculated to form a pterineprotein complex and functioning as a metabolic regulator by binding itself to DNA influencing RNA synthesis in insects (Cochran 1984).

8. Other nitrogenous compounds

The appearance of some unusual—both nitrogenous and non-nitrogenous substances, in varying amounts has been reported in several species of insect excreta (table 1). Some
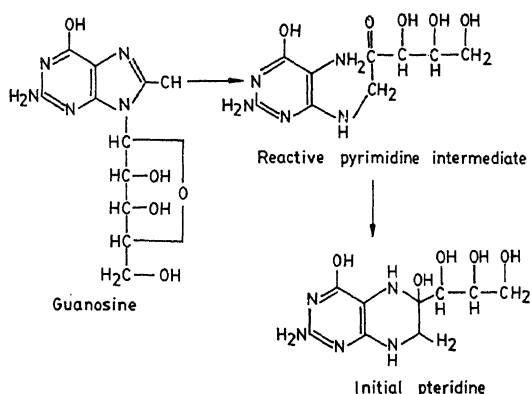
Figure 6. Conversion of purine into pteridine (adapted from Cochran 1975).
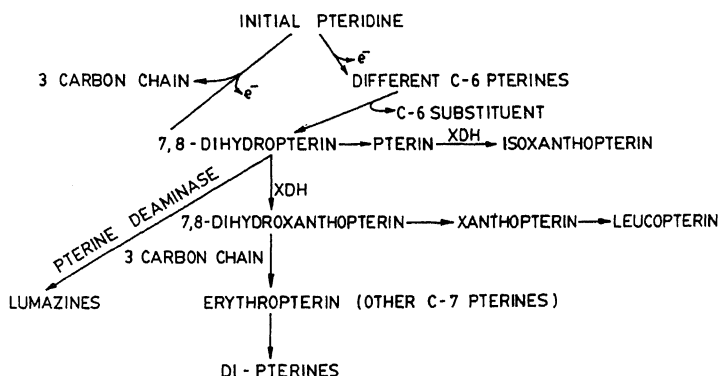
Figure 7. Generalized pterine biosynthetic pathway (adapted from Cochran 1975).

Table 1. Solubility of various nitrogenous compounds excreted by insects (Data adapted from Bursell 1967).

Nitrogenous end products	Nitrogen (%)	Solubility (mg/100 ml)
Hypoxanthine	41	70
Xanthine	37	260
Uric acid	33	6
Allantoin	35	60
Allantoic acid	32	Sparingly soluble
Urea	46	119,300
Ammonia	82	89,000
Arginine	32	15,000
Histidine	27	Moderately soluble
Cystine	6	11

of them are organic whereas the others are inorganic. The presence of creatine has been noted in *Lucilia* (Brown 1938a, b) and *Rhodnius prolixus* (Wigglesworth 1931) and of creatine and creatinine in *Tineola* (Powning 1953), *Anthonomous* (Mitlin *et al* 1964) and Apis (McNally *et al* 1965). In fact, even evidence for the conversion of creatinine to creatine in *Lucilia* larvae has been forwarded (Brown 1938b). Although judging from the very small quantities excreted, it is rather difficult to consider them as true excretory products, most of them have been proved to be of endogenous origin and as excretory products resulting from the metabolism of proteins (Gilmour 1961). Likewise, haematin has been detected in tsetse fly (Bursell 1967) and its origin has been traced to its blood feeding habit (Wigglesworth 1943).

The detection of some guanidine bases (Pant and Agrawal 1963) peptides (Harrington 1961) and proteins (Irreverre and Terzian 1959; Thayer and Terzian 1971; Mullins and Cochran 1973) in the excreta of some insects has been made. While in some cases, it is likely that these substances represent products of digestion correlated with their diet rather than nitrogen metabolism, much is yet to be known regarding their precise metabolic pathways of formation.

The presence of sugars and other carbohydrates in insect honeydews is also well recognized (Auclair 1963; Strong 1965). Although they have been considered as unabsorbed products of digestion in insects (Gray and Fraenkel 1954) or the excess absorbed voided, evidence has been forwarded by several investigators (Wolf and Ewart 1955; Ewart and Metcalf 1956; Bacon and Dickinson 1957; Mittler 1958; Sidhu and Patton 1970) to the effect that the excreted honeydews by insects, significantly differ qualitatively in carbohydrate content from that of the host plant. This evinces that insects are capable of excreting carbohydrates as metabolic end products.

The urine of some phytophagous insects has evinced the presence of some inorganic compounds like calcium carbonate, calcium oxalate and oxalic acid either in solution or in crystalline form (Takahashi *et al* 1969; Tiegler and Arnott 1972; Wigglesworth 1972). In addition, several other components like lipids in the honeydew of an aphid (Strong 1965), free fatty acids, sterols, triglycerides and many other unusual substances like juvenoid (Nemec and Jarolim 1980), have also been reported. While some of them have been considered to have originated from the host plant, a few appear to be excretory products.

In addition to the major excretory organs and the excretory substances voided by

them, the existence of minor excretory organs in insects has also been recognized (Wigglesworth 1972). They are of various types and occur in different parts of the insect body. Examples are, gut, hindgut, labial glands, utriculi majores, pericardial cells and nephrocytes. All these have been considered to possess excretory properties. The functions of these minor excretory organs have been reviewed by Maddrell (1971) and Riegel (1972) but the available information mainly deals with their physiology and ultrastructure. Their biochemical contribution to the process of excretion in insects is yet to be explored.

9. Concluding remarks

A perusal of the existing accumulated information about insects and their nitrogenous excretion makes it strikingly clear that each species within the same order and each insect within the same species and even the same insect during different developmental stages and, what is more, even the same stage at different time intervals in the self-same insect during its short life span, exhibits lability and diversity which characterize the class Insecta as such. They all present patterns of nitrogenous end products each different and varying from one another both qualitatively and quantitatively.

Consequently, the most glaring problem confronting the workers in insect biochemistry field is the probe into the biological significance of these diversities and the aspect of species biology wherewith they can be correlated.

The fact that different nitrogenous end products in varying amounts are excreted by different species of insects perhaps necessitates the re-assessment of the generalization that all terrestrial insects as a group are uricotelic. According to Razet's (1961) suggestion the group may be named based on the predominant constituent of the nitrogenous excretory product. Thus while the terms uricotelic, ureotelic and ammonotelic could be retained as is presently in vogue, those insects which excrete predominantly allantoin or allantoic acid need be appelled as allantoinotelic and allantoicotelic respectively. However, even this classification perhaps may not be able to stand long and may eventually need further changes as and when newer findings keep forthcoming and undoubtedly they will.

Elaborate studies have been made on the formation, nature and properties of several nitrogenous excretory products in insects and the mode of urine formation also has been probed in several insect species. However, the mechanism(s) or the mode of transfer whereby these end products find their way from the haemolymph into the main urine stream via the Malpighian tubules or any other tissue(s) is hardly understood. A model for uric acid transfer, though not a very convincing one, has been suggested but for other excretory products like the purines and the ureids that are voided by some insect species, one is just left conjecturing!

The process of recycling of water and ions by the Malpighian tubules-rectum complex and the very intricate processes involved in its regulation are other instances that still remain unclear. The process closely associated with osmoregulation and mediated by an active ion transport system in the Malpighian tubules causes the removal of waste material and in the process removes some useful metabolites as well from the haemolymph. Eventually, the latter are selectively reabsorbed and reclaimed in the rectum and the waste rectal fluid hypertonic with the haemolymph is subsequently excreted. Thus future research on osmoregulation

in insects will contribute to the knowledge in understanding the mechanisms underlying the excretion of nitrogenous products.

Another very interesting topic that needs attention is urate storage in insects. This was initially considered as an example of storage excretion i.e., a permanent accumulation of a major waste product in the insect body. Recent reports reveal these stored urates to be in dynamic equilibrium with active metabolic pools and under certain conditions capable of being mobilized and utilized by insects instead of being excreted. This utilization is mediated by the intracellular symbionts which metabolize the urates in the fat body where the storage often occurs. The fat body which plays a major role in the process of excretion in insects, has been considered comparable and equivalent to the liver in the mammals. Several biochemical aspects of this important tissue in insects are not still well understood. Examples are, the storage mechanisms, enzyme localization and the role of its symbionts in the metabolic processes occurring in it (fat body). Further, the recent claims on the presence of a urea cycle and occurrence of endogenous cellulases in phytophagous insects need be established.

It is hoped that future research on nitrogen excretion in insects will concentrate on the above mentioned unestablished or partially established phenomena observed in them with a view to gain a better understanding of these various processes occurring in them and their underlying mechanisms. Having in possession the presently available knowledge on the subject and the existing lacunae to be filled up and being equipped as we are today, with modern technology and delicate precision instruments, there is no reason why the gaps need remain any longer unbridged and insect biochemistry field branded as a blind alley!

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Cilia regeneration in cytochalasin B treated *Tetrahymena*

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Abstract. Cytochalasin B treated (20 μg/ml) and deciliated tetrahymenae consistently show an initial 1 h lag in cilia regeneration. The scanning electron microscopic observations reveal the altered cell morphology in cytochalasin B treated cells around this time. The possible interactions of cytochalasin B with deciliation induced protein and/or cytoskeletal matrix fibres for the observed delay in cilia regeneration kinetics and morphological alteration in *Tetrahymena* have been discussed.

Keywords. Cilia; regeneration; cytochalasin B; *Tetrahymena*.

1. Introduction

The ciliated protozoans have the unique property of regenerating their lost cilia. This regeneration process have been studied in some detail both at morphological as well as at biochemical level by experimental removal of cilia (Child 1965; Guttman and Gorovsky 1979; Bird and Zimmerman 1980; Hadley and Williams 1981; Calzone and Gorovsky 1982). It has been shown that colchicine, actinomycin D, puromycin, cycloheximide, dinitrophenol etc. can affect the cilia regeneration process (Child 1965; Rosenbaum and Carlson 1969; Rannestad 1974; Nelsen 1975; Keenan and Rice 1980). Conversely, it has been shown that deciliation interferes with cell cycle progression in *Tetrahymena* (Seyfert *et al* 1985). The present study is concerned with the effect of cytochalasin B (CCB) a well known fungal metabolite on the cilia regeneration process of *Tetrahymena*. CCB has been shown to profoundly affect diverse cellular functions like cell motility, morphology, cell division etc. in variety of cell types (Carter 1967; Copeland 1973).

2. Experimental

2.1 *Cell culture and cilia amputation procedure*

Tetrahymena pyriformis (W) was cultured axenically in 2% proteose-peptone supplemented with 0·02% liver extract at 24° ± 1°C. Healthy and actively swimming cells from log phase cultures were deciliated according to the method of Rosenbaum and Carlson (1969). Immediately after deciliation procedure, the cell suspension is centrifuged for 2–3 min, supernatant is decanted of and cells resuspended in original growth medium.

2.2 *CCB treatment*

CCB was used at a concentration of 20 μg/ml (prepared from a 10 mg/ml DMSO stock solution) which was found to be optimum dose tolerated by this ciliates. The

cells were treated with CCB for 1 h and then after several wash with phosphate buffer were resuspended in culture medium. Survival of the drug treated cells was determined by dye exclusion test (0·1% trypan blue). The following schedules were adopted for duration and time of CCB treatment:

(i) Cells were only pretreated with CCB before cilia amputation.
(ii) Cells were pretreated as well as posttreated with CCB throughout the cilia regeneration period.
(iii) Cells were CCB-treated for 1 h immediately after deciliation.
(iv) Cells were only post-treated throughout the cilia regeneration period.

The cilia regeneration timing in both control and treated cells were monitored by haemocytometer counting and was calculated from cell motility index. All experiments were performed atleast 4 times.

2.3   *Light microscopy*

The control and treated cells were routinely fixed with acetic acid ethanol (1 : 3) on subbed slides prepared with 0·5% gelatin and stained either with buffered glemsa solution or negatively stained with 10% nigrosin solution. Stained cells were examined under a Carl Zeiss Amplival Microscope.

2.4   *Scanning electron microscopy*

Normal, deciliated and CCB-treated cells were prefixed with karnovsky's (1965) fixative for 1 h, rinsed with cacodylate buffer (pH 7·6) and then post-fixed in ice-cold osmium tetroxide for 1 h. Fixed cells were washed with cacodylate buffer and then passed through graded series of ethanol and finally through two changes with acetone. Cells were thinly spread on cover glass; lypholized for 15–20 min and then placed on metallic stubs. The specimens were coated with gold in vaccum and observed in a Cambridge stereoscan S4-10 operated at 30 kV.

3.   **Results and discussion**

The cilia regeneration timing was monitored in deciliated *Tetrahymena* and was calculated from the cell motility index. It was found that ca. 50% cell motility occurred around 2 h after cilia amputation and 90% motile cells were observed at 5 h. The light and scanning electron microscopic analyses of cell surfaces of cilia regenerating tetrahymenae at different periods of time also confirmed these findings. These timings were, however, somewhat different from which was reported by Rosenbaum and Carlson (1969) and Guttman and Gorovsky (1979) where they observed a complete ciliogenesis by 90 min. When CCB was administered for 1 h to tetrahymenae, immediately after removal of the cilia, there was an inhibition of cilia regeneration for 1 h. However, from 2 h onwards, the treated cells gradually recover from this initial delay and by 5 h ca. 90% cells regenerated their cilia (figure 1). The treatment with CCB prior to or 1 h after deciliation had no effect on cilia regeneration timings.
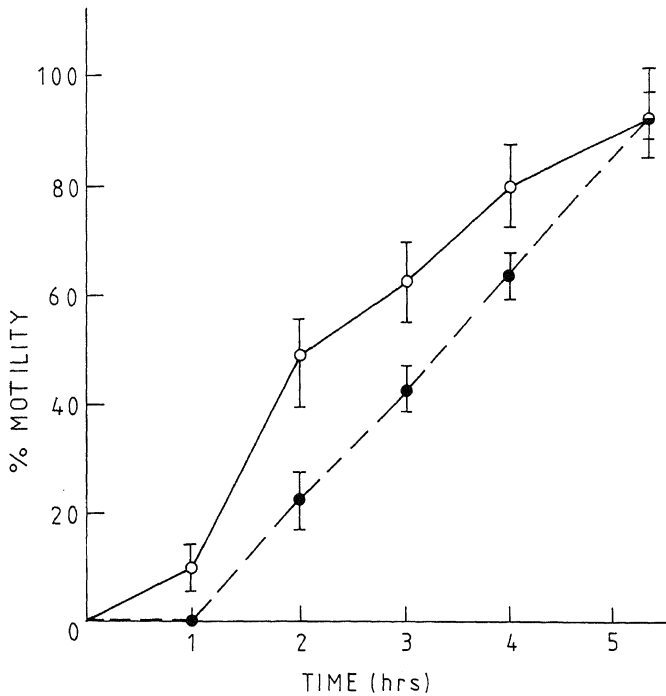
Figure 1. The effect of CCB on cilia regeneration timing in *Tetrahymena pyriformis*. The figure depicts the summary of 4 experiments (○), Control; (●), treated. Bars indicate SE $(S/\sqrt{n})$.

In many cases (ca. 30% of the deciliated and CCB-treated cells observed under scanning electron microscope) CCB treatment produced a remarkable alteration in cell morphology. The drug treated ciliates often showed a twisted contour at initial period (between 0·5–1 h) after deciliation and as a result the arrangement of kinetics (ciliary rows) appeared to be distorted (figure 2). In control cells such aberrant morphological feature was not encountered. Further, DMSO treatment alone had neither any effect on timing of cilia regeneration nor on morphology of the deciliated tetrahymenae.

It was shown earlier that the synthesis of 80,000 dalton protein (deciliation induced protein, DIP) began shortly after the tetrahymenae were deciliated and declined around 1 h after deciliation. Whereas the synthesis of tubulin (55,000 dalton protein) could be detected only 1 h after deciliation (Guttman and Gorovsky 1979). The possible presence of a specific initiation protein factor(s) in the ciliary morphogenesis was also suggested (Hadley and Williams 1981). Thus, it might be tempting to speculate that CCB which is effective only during the first hour after deciliation might be interfering with the DIP proteins and/or initiator protein(s), thereby causing a lag in the cilia regeneration timing.

CCB is known to produce alteration in cell morphology as has been reported in fibroblasts, myoblasts, chick mesoderm, coelomocyte, $3T_3$ cells etc. (Carter 1967; Sanger and Holzer 1972; Edds 1980). It also produces many adverse effects on microfilaments. It blocks the polymerization and causes disorganization of actin
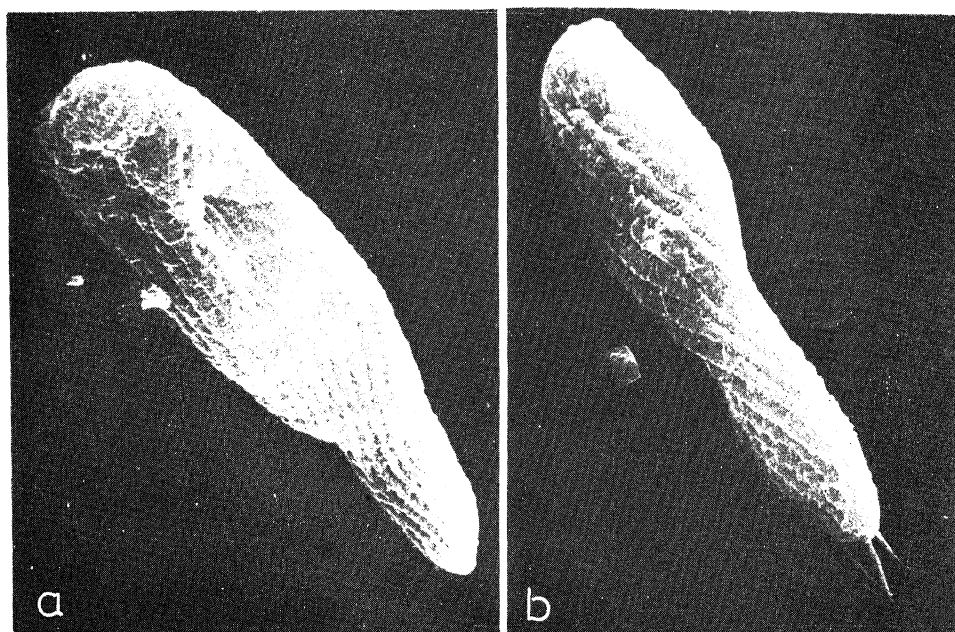
Figure 2. a. Deciliated *T. pyriformis* ($\times$ 8505). **b.** CCB-treated and deciliated *T. pyriformis* ($\times$ 8551).

filaments (Brown and Spudich 1979; Fox and Philips 1981). It has recently been indicated that the epiplasm layer below the cell surface of *Tetrahymena* might contain actin filaments (Williams and Vaudaux 1979). It is plausible that in CCB-treated cells, there might be a disruption in the cytoskeletal matrix leading to a change in the cortical architecture affecting the spatial arrangement of kinety. This, in turn, might also lead to the delay in cilia regeneration process.

Acknowledgement

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Haemolymph protein profiles during the gonadotrophic period of *Gesonula punctifrons* Stal. (Orthoptera: Insecta)

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Abstract. Quantitative and qualitative aspects of the haemolymph proteins during the entire life span of both sexes of *Gesonula punctifrons* Stal. in relation to their variations during gonadotrophic period are discussed. The appearance of a female-specific protein in 8-day old female which was absent in freshly-emerged and senescent females and in males, is of interest.

Keywords. *Gesonula punctifrons*; protein profiles; gonadotrophic period.

1. Introduction

Haemolymph proteins in female insects are known to undergo quantitative and qualitative changes correlated with egg maturation (Hill 1962; Engelmann and Penney 1966). The fluctuations in yolk formation, as well as haemolymph concentration in relation to gonad maturation in both sexes, are believed to reflect the net balance between the variation in the rate of protein synthesis in the fat body and protein uptake by developing oocytes. The haemolymph protein pattern of male insects though more or less static, nevertheless shows variations in relation to the physiological state of the individuals. The female insect utilizes the available haemolymph protein for vitellogenesis (Engelmann 1970). Quantitative and qualitative differences in the haemolymph protein pattern existing between male and female insects, as well as, haemolymph protein pattern of female correlation of oocyte development (Engelmann and Penney 1966; Thomas and Nation 1966) have been confirmed through present observations on *Gesonula punctifrons*.

2. Materials and methods

The acridids used for the present studies were taken from a stock culture maintained in the laboratory. The insects were fed *ad libitum* on *Eichhornia crassipes* leaves. Haemolymph for quantitative studies and also for electrophoresis was drawn into a capillary tube from a puncture made on the cervical region and also by amputating the antennae. The samples were centrifuged at 10,000 g for 30 min. The clear supernatant was used as protein source for electrophoresis. Protein was quantitatively estimated by the method of Lowry *et al* (1951). For the qualitative profile of the protein, polyacrylamide gel electrophoresis was carried out, employing the method of Davis (1964). The gels were removed from their tubes and stained with Commassie brilliant blue in a mixture of methanol, acetic acid and water in the ratio of 25:7:68. After destaining, the gels were scanned using LKB 2202 Ultrascan laser densitometer. Haemolymph of each sample was repeated thrice and identically resolving protein fractions were ordered according to their increasing mobility as illustrated in figures 1 and 2.

3. Results

Figure 1 relates to the quantitative protein concentration in the haemolymph during the entire life span of normally reproducing female and male insects, and the corresponding oocyte length. The total protein content of haemolymph in freshly-emerged female was 23 μg/ml and increased to a maximum of 48·9 μg/ml on 14th day subsequently declining to 21 μg/ml on the 26th day. During the 2nd gonadotrophic period (28–44th day), an upward trend in haemolymph protein concentration from 28th to 36th day 31·2 μg/ml and dropped to 21·5 μg/ml on 44th day. From 44th day (second oviposition) to 52nd day (senescent period), the haemolymph protein level gradually declined to 10·5 μg/ml. The haemolymph concentration of male was 12·1 and 10·2 μg/ml on the 1st and 38th day respectively, and did not show drastic variations. Oocyte length varied from 1–4·8 mm (6–20th day; 1st oviposition) and 0·8–4·5 mm (28–44th day: 2nd oviposition). During the final stages of maturation (5·2–5·8 mm), the total protein content in haemolymph declined rapidly.

A comparison of densitometric scans of haemolymph proteins (figure 2) revealed interesting results. The newly emerged female (0 day) showed the presence of two major fractions, and as the oocyte maturation progressed, a new fraction with R_m value 0·221 (table 1) appeared and reached its peak on the 8th day. A sudden drop in the concentration of this protein fraction starting from the 12th day was clearly evident, and was almost eliminated after oviposition. Newly emerged and senescent females were completely devoid of such a protein fraction, and surprisingly males did not show the presence of this fraction throughout their life span.

4. Discussion

Cyclic fluctuations in the haemolymph protein concentrations of normal *G. punctifrons* can be correlated with the egg maturation process as in other species (Hill 1962;
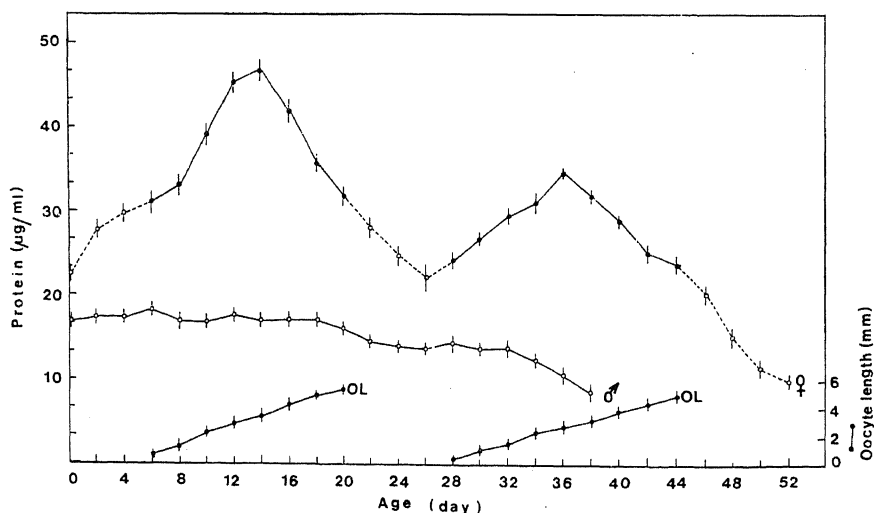
Figure 1. Quantitative haemolymph protein profiles and oocyte length of *G. punctifrons* (OL, oocyte length).

F - Female; AEL - After egg laying
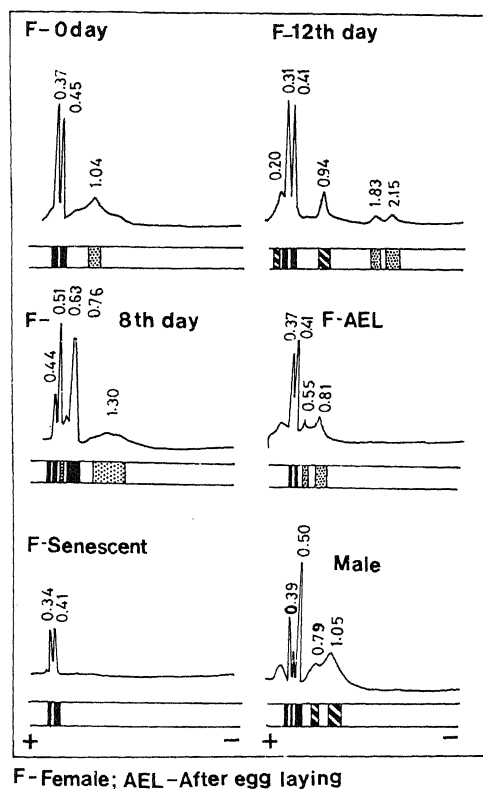
Figure 2. Densitometric scan of protein fractions of *G. punctifrons*.

Table 1. Electrophoretic analysis of the haemolymph protein profile of *G. punctifrons* during gonadotrophic period.

Protein Ef	A 0·115	B 0·136	C 0·163	D 0·178	E 0·221	F 0·242	G 0·273	H 0·288	I 0·305	J 0·420	K 0·696	L 0·778
Stage/day												
Female												
0	28·391	20·843	50·766	—	—	—	—	—	—	—	—	—
8	13·648	—	17·981	0·878	53·899	—	—	—	—	13·774	—	—
12	—	—	12·754	—	32·520	28·852	—	—	—	14·686	4·485	6·230
Oviposited	—	—	—	—	—	—	7·097	7·719	1·446	83·738	—	—
Senescent	31·094	68·906	—	—	—	—	—	—	—	—	—	—
Male	1·395	0·720	64·874	33·011	—	—	—	—	—	—	—	—

Values represent area percentage.

Engelmann 1970; Gillott and Elliott 1976). However, the lesser protein concentration during the freshly-emerged female and 2nd day of adult life does not appear to be related to oocyte development because the oocytes are not competent to sequester yolk at this time. Hill *et al* (1968) suggested that haemolymph protein may be used for somatic growth in *Schistocerca gregaria* and large amounts of proteins were deposited in the cuticle and flight muscles during the somatic growth phase. Once

yolk deposition begins, a significant increase in the haemolymph protein concentration indicates that synthesis greatly exceeds uptake by the oocytes. However, during the final stages of vitellogenesis, the converse appears to be true as protein levels in the haemolymph are drastically reduced.

In *G. punctifrons* at oocyte length of 3–4 mm, protein concentration was 31·2 μg/ml and at the 4–4·8 mm stage, the maximum protein content of about 48·9 μg/ml was reached. Later, though the development of oocytes progresses protein concentration in the haemolymph declines. Similar fluctuation in protein concentration in the haemolymph was observed by Gillott and Elliott (1976) in *Melanoplus sanguinipes*. Scheurer and Leuthold (1969) have also suggested that in *Leucophaea maderae* the first and subsequent gonadotrophic periods are of sufficient similarity and they may be treated together. However, in *G. punctifrons* protein concentration in haemolymph is comparatively lower during second gonadotrophic period. In oviposited and senescent females, a further decrease in the concentration of the protein was noticed.

In *G. punctifrons*, formation of yolk starts after the age of 6 days (after previtellogenic period). If fluctuation in the concentration of protein fractions and the formation of yolk is correlated, the concentration of protein in the haemolymph falls when the yolk formation starts and the protein accumulates in the ovary. More protein accumulates in the haemolymph during production of the first batch of eggs. This is because of low food intake and stored energy materials are already utilized.

In *G. punctifrons* the qualitative protein profiles vary during development. The same trend was noticed in *Schistocerca gregaria* by Hill (1962). Further, he has reported that a sex-specific or vitellogenic or female specific protein is utilized during vitellogenesis. In *G. punctifrons* also, a sex-specific protein is evident in 8th day old female. In the present study except for the absence of female-specific protein, the pattern of mature males closely resembles that of vitellogenic female. This is in agreement with the report of Elliott and Gillott (1979) in *M. sanguinipes*.

Acknowledgements

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Polyol dehydrogenases in the eggs of the silkworm *Bombyx mori* L.

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Abstract. Activities of some of the dehydrogenases involved in the formation of polyols were examined in diapause, non-diapause and acid treated artificial non-diapause eggs. The studies revealed that NADP-SDH may be important in the production of sorbitol during the onset of diapause and that NAD-GPDH may be playing a more important role than NADP-GDH in the production of glycerol.

Keywords. *Bombyx mori*; dehydrogenases; sorbitol; glycerol.

1. Introduction

A specialised conversion of glycogen to polyhydric alcohols was first demonstrated in the diapause embryo of *Bombyx mori* by Chino (1957a). Two polyols namely sorbitol and glycerol were shown to accumulate in diapause eggs while glycogen content decreased (Chino 1957b, 1958). Many studies have shown that this breakdown of glycogen to form polyols is closely correlated with the onset of diapause in silkworm eggs (Chino 1957a; Yaginuma and Yamashita 1977, 1978).

The enzyme polyol dehydrogenases are involved in the conversion of glycogen to polyols. The presence of these enzymes in the haemolymph of silkworm was demonstrated by Faulkner (1958). Later studies (Chino, 1960; Yaginuma and Yamashita, 1979) demonstrated the presence of the following polyol dehydrogenases in silkworm eggs.

(I) NADP dependent sorbitol dehydrogenase.
(II) NADP dependent glycerol or sorbitol-6-phosphate dehydrogenase.
(III) NAD dependent glycerol phosphate dehydrogenase.
(IV) NAD dependent sorbitol dehydrogenase.

While I and II enzymes were suggested to be playing an important role during the onset of diapause the IV one has been suggested to be playing an important role during the termination of diapause. The significant observation of all the earlier workers is that all these dehydrogenases are present in non-diapause eggs also where polyol formation never occurs. The present studies were undertaken to re-examine the activities of polyol dehydrogenases in diapause eggs following the onset of diapause and during embryonic development in non-diapause as well as artificial non-diapause eggs where diapause has been interrupted using HCl treatment. The activities of polyol dehydrogenases namely NADP dependent sorbitol dehydrogenase (NADP-SDH), NADP dependent glycerol dehydrogenase (NADP-GDH) and NAD dependent glycerol phosphate dehydrogenase (NAD-GPDH) have been studied.

2. Materials and methods

Bivoltine (NB_4D_2) and multivoltine (pure Mysore) races of the silkworm *Bombyx mori* L. were maintained under standard conditions. Eggs laid on polythene sheets were kept at $25 \pm 2°C$ with relative humidity 75%. For breaking diapause, 20 h old eggs were treated with HCl (Sp. gr. 1·075) at 46·1°C for 3–4 min, washed thoroughly with water and kept at $25 \pm 2°C$.

2.1 *Enzyme preparation*

A 10% (w/v) homogenate of the eggs was prepared using a glass homogeniser fitted with Teflon pestle. The homogenate was filtered through a cotton pad and centrifuged at 5500 *g* for 15 min at 0°C. The supernatant was filtered through Whatman No. 1 filter and the resultant filtrate was used as the enzyme source.

2.2 *Assay of the enzyme activity*

NADP-SDH activity was determined following the method of Takahashi *et al* (1974). The reaction mixture contained 50 mM Tris-HCl buffer of pH 7·5, 3·3 mM fructose, 0·33 mM NADPH and 0·1 ml enzyme extract in a final volume of 1 ml. NADP-GDH activity was determined based on the method of Faulkner (1958). The reaction mixture consisted of 20 mM Tris-HCl buffer of pH 7·5, 4 mM $MgSO_4$, 10 mM dihydroxy acetone, 0·07 mM NADPH and 0·1 ml enzyme solution in a final volume of 1 ml. NAD-GPDH activity was determined based on the method of Baranowski (1949). The reaction mixture consisted of 20 mM Tris-HCl buffer of pH 8·5, 4 mM $MgSO_4$, 10 mM dihydroxyacetone phosphate, 0·07 mM NADH and 0·1 ml of the enzyme extract in a final volume of 1 ml.

The reactions were initiated by the addition of respective substrates. The enzyme activity was determined by measuring the optical density at 340 nm. One unit of the enzyme activity was defined as the amount causing decrease of optical density by 0·01/min. Protein content was determined according to Lowry *et al* (1951) using bovine serum albumin standards.

3. Results

3.1 *NADP-SDH activity*

This activity could be detected in the extracts of both diapause and non-diapause eggs. In non-diapause eggs, though sorbitol is never known to accumulate during embryogenesis, NADP-SDH activity was considerably high at the time of oviposition. The level of activity remained constant throughout the embryonic development (figure 1). In diapause eggs, the activity was higher than in the non-diapause eggs at the time of oviposition. The activity further increased on the first day. But for a small decrease on the second day, the activity remained high upto the 5th day following oviposition which corresponds to the period of rapid glycogen break down in diapause eggs. Following 5th day, the activity decreased as the age increased and it was relatively low by 30 days. In acid-treated artificial non-diapause
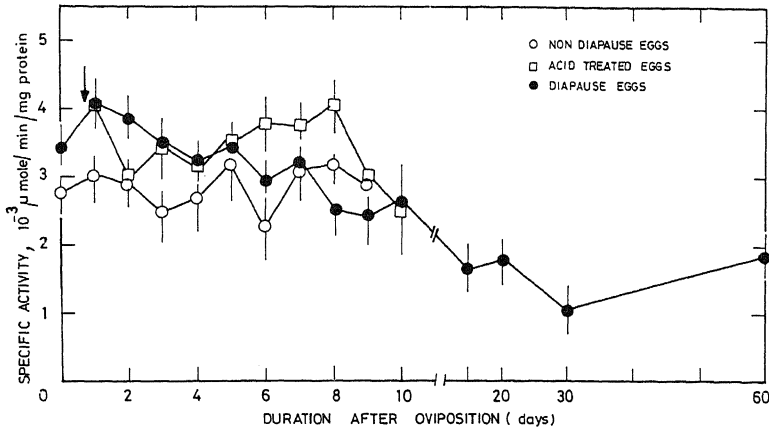
Figure 1. Changes in NADP-SDH activity during diapause and embryogenesis. In the present and all the following figures, Mean values (n = 4) along with SD are plotted and arrow indicates acid treatment.

ggs, the activity of the enzyme decreased significantly following acid-treatment. hough the activity was relatively higher than in the non-diapause eggs, it remained onstant as in the case of the latter.

2 *NADP-GDH activity*

he activity was quite high immediately after oviposition in non-diapause eggs. The ctivity further increased on the second day following which it progressively ecreased as embryogenesis progressed (figure 3). In diapause eggs, the activity attern was very much comparable to that observed in non-diapause eggs upto the 0th day. But following this period, the activity increased significantly. In artificial on-diapause eggs, the activity followed a pattern very similar to that observed in the on-diapause eggs.

3 *NAD-GPDH activity*

n non-diapause eggs, the activity was quite high immediately after oviposition and it ecreased by the second day following which it increased as embryogenesis rogressed and decreased just before hatching (figure 4). The activity was found to be igher in diapause eggs at the time of oviposition. It increased subsequently but a ignificant change was noticed after the 10th day when the activity increased very nuch. In acid-treated eggs, the activity resembled that seen in non-diapause eggs.

. Discussion

he studies of Chino (1960) and Takahashi *et al* (1974) showed that NADP-SDH is ctive throughout the egg life but there was no change in its activity that could be trongly correlated with diapause especially its termination. Secondly, since this

enzyme was found to be equally distributed in both the diapause and non-diapause eggs, it was suggested that this enzyme plays no specific role in sorbitol formation during diapause. NAD dependent sorbitol dehydrogenase has been suggested to be playing an important role during termination of diapause (Yamashita *et al* 1981). Hence, the role of polyol dehydrogenases in the formation of sorbitol during the initiation of diapause is not clear. Present studies showed that in diapause eggs, the activity of NADP-SDH is much higher than in non-diapause eggs. Secondly, immediately after oviposition, a sudden but brief increase in the activity was registered during the second and third days in diapause eggs which actually corresponds to the rapid glycogen break down phase (Chandrashekar and Geethabali 1987). Interestingly, a similar increase in the activity of this enzyme was also recorded (figure 2) by Yaginuma and Yamashita (1979). Such an increase is not observed in non-diapause eggs. Also, the activity somewhat decreased, immediately following acid treatment to reach the level characteristic of a non-diapause egg on the second day. Thus, clearly, there is no increase in the activity of NADP-SDH accompanying the early embryonic development suggesting that the enzyme may be playing an important role atleast during the onset of diapause. While phosphorylase 'a' is said to be the key enzyme involved in providing the initial substrate for polyol formation (Chandrashekar and Geethabali 1987), NADP-SDH may be important in the actual formation of sorbitol.

The increase in the activity of NADP-GDH in diapause eggs 10 days following oviposition indicates that it may be contributing towards the formation of glycerol. However, compared to the level of activity observed at the time of oviposition, this increase is not very significant. Since (i) the enzyme is equally active in both diapause and non-diapause eggs, (ii) there is no change in its activity correlated with the onset of diapause and (iii) 'no significant change was seen as a result of acid-treatment, it can be said that though this enzyme may contribute towards the formation of glycerol, it may not be a key enzyme.
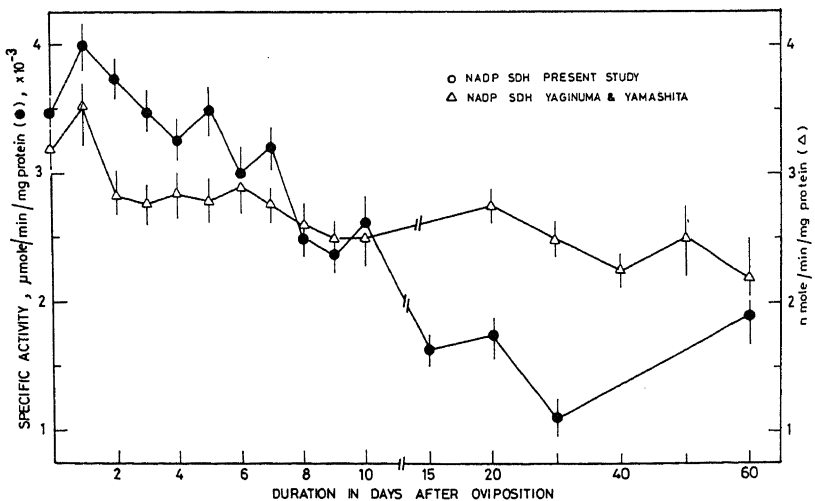
Figure 2. Changes in NADP-SDH activity during diapause. Results from Yaginuma and Yamashita (1979) are presented for comparison. Note the increase in the activity in both the cases on the first day following oviposition.
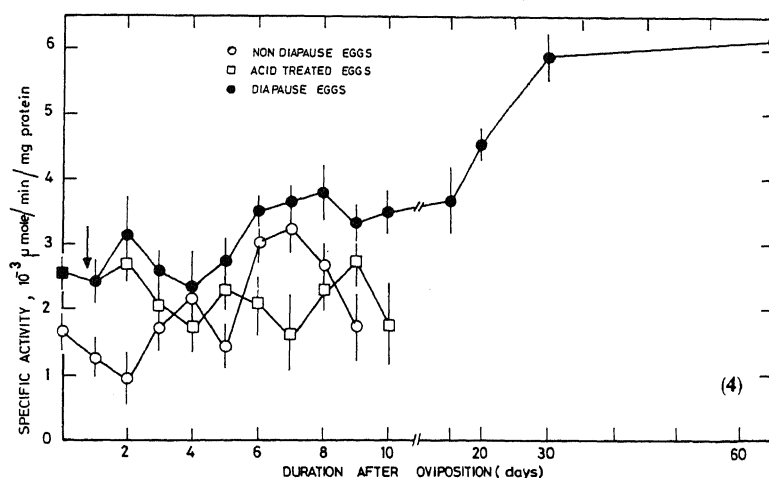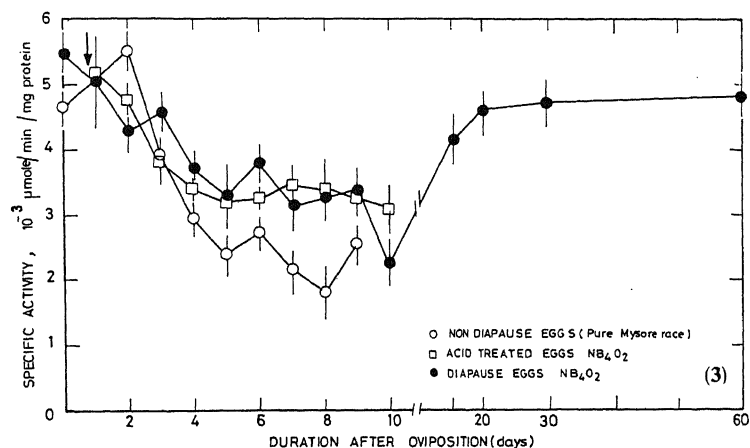
Figures 3 and 4. Changes in (3) NADP-GDH and (4) NAD-GPDH activity during diapause and embryogenesis.

NAD-GPDH was found to be quite active in both diapause and non-diapause eggs. The activity increased as embryogenesis progressed especially around periods corresponding to blastokinesis and blue egg stages. Hence, it may be important in embryonic development as already suggested by earlier workers (Chino 1960; Horecker, 1968; Yamashita *et al* 1981). In diapause eggs, not only the activity was much higher than that observed in non-diapause eggs, it increased slowly following oviposition up to the 10th day and rapidly thereafter. This closely corresponds to the initial slow increase in the glycerol level followed by its rapid accumulation in diapause eggs (Yaginuma and Yamashita 1978; Geethabali and Chandrashekar 1987). Hence, NAD-GPDH may be playing a more important role than NADP-GDH in the production of glycerol during diapause. However, not much is known about the conversion of glycerophosphate to glycerol in silkworm eggs. This would involve the removal of phosphate either by a specific glycerophosphatase or other general phosphate cleaving enzyme. At this juncture, it can be said that the high activity of NAD-GPDH would lead to the accumulation of glycerophosphate in

silkworm eggs which is a phenomenon common in many insects during diapause or even anaerobiosis (Chefurka 1965). If NAD-GPDH activity is accepted to be contributing mainly to the formation of glycerol, it can be realised that the NADP requirement for glycerol formation is minimised.

Acknowledgement

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fluence of precocene-I on the development of vitellogenic oocytes in rogoderma granarium Everts (Coleoptera: Dermestidae)

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Abstract. Female *Trogoderma granarium* pupae, 0–96 h old, were treated with precocene-I at 6 h intervals. Reduction in the vitellogenic oocyte number was observed in adults after precocene treatment at specific ages. Precocene-I was effective when applied to 6, 18, 30, 42, 54 and 90 h old pupae while in 0, 12, 24, 36, 48, 60, 66, 72, 78, 84 and 96 h old pupae this compound was not significantly effective. Corpus allatum activity is a major contributory factor towards precocene susceptibility. The results suggest that pupae at different ages exhibit periodic changes in corpus allatum activity that might be related to cycles of development in the reproductive system.

Keywords. Precocene-I; vitellogenic oocytes; *Trogoderma granarium*.

Introduction

ecocenes are known to block juvenile hormone secretion in a number of insects owers *et al* 1976). In addition to inducing precocious development in immature ges, precocene has also been shown to produce sterilization effects in certain nale insects (Bowers 1976; Bowers *et al* 1976; Bowers and Martinez-Pardo 1977; asner *et al* 1979; Belles and Messeguer 1981; Farag and Varjas 1981; Martinez rrau *et al* 1981; Zamorano *et al* 1981; Samaranayaka-Ramasamy and aaudhury 1982; Wilson *et al* 1983; Bitsch and Bitsch 1984; Belles *et al* 1985). nfortunately precocene is without effect against most holometabola and is active ly on very few sensitive genera of Heteroptera and Acrididae (Masner *et al* 1979; ner *et al* 1981; Pratt *et al* 1981; Alrubei 1986). In susceptible insect species, ecocenes act as anti-allatins or allatocidal agents causing atrophy and necrosis of e corpora allata (Bowers and Martinez-Pardo 1977; Pener *et al* 1978; Schooneveld 79a; Feyereisen *et al* 1981; Soderlund *et al* 1980; Pratt *et al* 1980; Miall and ordue 1980; Hales and Mittler 1981; Bowers and Feldlaufer 1982; Pratt 1983; Pratt d Pener 1983; Lange *et al* 1983; Raynaud *et al* 1985). In *Locusta migratoria* chooneveld 1979a) and *Oncopeltus fasciatus* (Masner *et al* 1979) the destruction of e corpora allata is irreversible. In less susceptible species, however, the functioning the glands is only temporarily inhibited (Feyereisen *et al* 1981; Belles and esseguer 1981; Wilson *et al* 1983). It is also claimed that precocene is only effective nen the corpora allata are active. At present, the hypothesis is based upon physio-gical evidence obtained *in vivo* which may be coincidental (Pratt and Pener 1983). Although the mode of action of these active compounds is yet to be clarified, there opears to be some evidence that precocenes act directly upon the corpora allata owers and Martinez-Pardo 1977; Pratt 1983). Accordingly, such induced titer sturbances of juvenile hormone in precocene treated female insects could result in e disruption of normal reproductive cycles. In the tsetse fly *Glossina morsitans* orsitans, precocene treatment of the female sterilized her female offspring

(Samaranayaka-Ramasamy and Chaudhury 1982). While in the milk weed bug *O. fasciatus*, cessation of ovarian development and resorption of oocytes occur (Unnithan *et al* 1977) or vitellogenesis is inhibited (Masner *et al* 1979). In *Drosophila melanogaster*, precocenes reduced the number of vitellogenic oocytes present in a dose-dependent manner (Wilson *et al* 1983). In a variety of adult insects precocene treatment soon after emergence prevents ovarian development (Bowers 1976). As *Trogoderma granarium* adults do not feed and are short-lived, maturation of the reproductive organs takes place during the pupal stage. On emergence, the females have fully differentiated vitellogenic oocytes (Karnavar 1972, 1973).

The present investigation was carried out to find out whether the female *Trogoderma* (Coleoptera: Dermestidae) exposed to precocene-I at various stages of pupal life would show any specific changes in the vitellogenic oocyte number and also attempted to characterize the inhibitory effect of precocene on the corpora allata function.

2. Materials and methods

Pupae of *T. granarium* were obtained from larvae maintained on crushed wheat at $35 \pm 1°C$. In any set of experiment, the control and experimental pupae were obtained from the same culture. Pre-pupae could be separated from larvae, as they roll slightly and lie on their back, remaining motionless for a few hours. Later, they stretch their body and the larval cuticle splits along the mid-dorsal line. This indicates the initiation of pupation (0 h). Such pupae were collected and sexed. Female pupae of the ages 0–96 h at 6 h intervals were utilized for the present study. Synthetic precocene-I-7 methoxy 2, 2-dimethyl-chromene (Sigma Chemicals company, USA) was dissolved in acetone and 0·1, 0·2, 0·5 and 1 μg per pupa was applied topically with a Hamilton microsyringe. Control pupae were treated with acetone. All experiments were carried out at least in 5 replicates. After treatment, the pupae were maintained at 35°C in separate containers till adult emergence.

Ovaries were dissected from adult females within 24 h of emergence. The ovarioles were gently teased apart and the number of vitellogenic oocytes was determined. As small adults produce lesser number of eggs (Karnavar 1972) pupae of the same size and weight were used in experimental and control batches.

3. Results

The results obtained showed that the pupae were more susceptible to precocene-I treatment at 6, 18, 30, 42, 54 and 90 h of age (table 1) compared to 0, 12, 24, 36, 48, 60, 66, 72, 78, 84 and 96 h.

Decrease in the number of vitellogenic oocytes is expressed as percentage of reduction in figure 1. It was fairly high (32·3, 25·34 and 23·76) for a dose of 0·1 μg precocene-I in 30, 54 and 90 h old pupa. Percentage reduction obtained after application of 0·2 μg/pupa on 6, 18, 30, 42, 54 and 90 h old pupae was 18·31, 6·02, 19·77, 22·56, 34·04 and 26·04. The doses 0·5 and 1 μg precocene-I yielded higher reduction percentages. At the dose 0·5 μg/pupa it was 31·84, 32·1, 28·85, 39·19, 34·32 and 25·65 and 16·67, 33·38, 20·43, 16·30, 36·82 and 25·21 at the 1 μg/pupa level.

At 0·2, 0·5 and 1 μg/pupa, the vitellogenic oocyte number showed significant variation at 12 h intervals. Pupae of 6, 18, 30, 42 and 54 h old, on treatment with

Table 1. Showing the effect of precocene-I on the number of vitellogenic oocytes in *Trogoderma granarium*.

Age of pupae	0·1 μg/pupa		0·2 μg/pupa		0·5 μg/pupa		1 μg/pupa	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
			Number of vitellogenic oocytes					
6	70±13·40	72·18±10·87	66±4·50	80·8±9·96	54·8±15·20	80·4±11·84	61·0±8·49	73·2±9·64
18	61·2±8·15	67·4±5·37	65·6±6·99	69·8±13·31	55±10·22	81·0±8·91	51·65±10·67	77·53±6·85
30	43·6±11·48	64·4±10·48	56·8±12·49	70·8±26·40	52·86±13·13	74·29±8·56	62·33±12·45	78·33±11·04
42	66·86±8·93	69·14±10·62	54·43±11·03	79·29±12·02	41·5±8·76	68·25±5·86	54·22±13·70	64·78±21·96
54	55·25±13·56	74·0±8·24	52·33±13·48	79·33±5·37	52·33±8·19	79·67±4·69	50·8±8·90	80·4±4·8
90	61·33±12·47	80·44±10·90	50±5·05	67·6±5·42	68·4±4·44	92·0±5·51	49·14±8·67	65·71±8·03
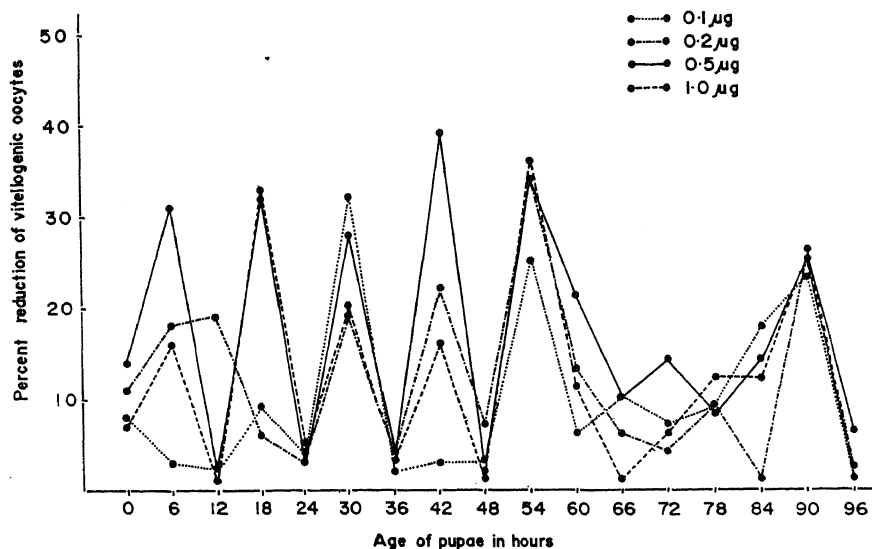
Figure 1. Percentage reduction in vitellogenic oocyte number in precocene-I treated
T. granarium.

precocene-I yielded adults which showed significant reduction in oocyte number.
Pupae of 90 h old also appeared to be susceptible to precocene-I treatment whereas
0, 12, 24, 36, 48 and beyond 54–84 h pupae were not affected by the precocene-I
treatment. Even at the lowest dose used (0·1 μg/pupa) 30, 54 and 90 h pupae showed
significant response to precocene-I treatment.

4. Discussion

From the results obtained it is clear that precocene-I treatment induces reduction in
the oocyte number at specific ages in *T. grganarium.* In this investigation, disruption of
normal oocyte development is observed in 6, 18, 30, 42, 54 and 90 h old treated
pupae. Precocenes are known to produce antigonadotrophic effects in certain insect
species through a mechanism involving corpus allatum inactivation (Bowers *et al*
1976). For instance, treatment of female *O. fasciatus* larvae with precocene-II induces
precocious metamorphosis and sterility (Unnithan *et al* 1977). Topical or contact
treatments of freshly moulted adults of *O. fasciatus* have resulted in a strong blockage
of vitellogenesis prevention of ovarian development and eventual oocyte resorption
(Unnithan *et al* 1977). Precocene-II applied to adults and larvae of *Dysdercus
cingulatus* significantly reduces fertility of both sexes (Farag and Varjas 1981). In
B. germanica some of the adult females treated with precocene-II show a delay in the
formation of the first ootheca and a broad variety of deficiencies in its formation.
Continued observations showed progressive improvements in the formation of
successive oothecae indicating that corpus allatum inactivation is only partially
achieved (Belles and Messeguer 1981). In *D. melanogaster* precocenes reduced the
number of vitellogenic oocytes when applied to either newly eclosed or gravid
females (Wilson *et al* 1983).

The secretion of juvenile hormone by the corpus allatum is under the control of the brain. This hormone acts at the genetic level being involved in the transcription of specific mRNAs. Juvenile hormone is known to stimulate fatbody synthesis of vitellogenin and oocyte maturation of female insects (Adams 1970; Dogra and Ewen 1970; Engelmann 1970, 1976, 1979; Wigglesworth 1970; Gwadz and Spielman 1973; Mc Caffery 1976; Buhlmann Georges 1976; Kelly and Davenport 1976; Postlethwait *et al* 1976; Schooneveld *et al* 1979; Kozhanova and Chudakova 1985). Biological effects of precocene on insects result from induced juvenile hormone deficiency caused by the cytotoxic action of the compound on corpus allatum cells (Feyereisen *et al* 1981; Schooneveld 1979 a, b; Unnithan *et al* 1977). The effects of precocene-II on ovarian growth of *O. fasciatus* can be reversed by the application of exogenous juvenile hormone (Unnithan *et al* 1977). In *D. melanogaster*, since juvenile hormone is required for vitellogenin uptake by the oocytes, it is suggested that precocene reduced the level of endogenous juvenile hormone leading to reduction in the number of vitellogenic oocytes in treated females (Wilson *et al* 1983). The reduction in the number of vitellogenic oocytes found in the present study is consistent with the antigonadotrophic effects of precocene reported and is possibly affected by depression of haemolymph juvenile hormone titres as in other insects.

Variations in the response between *Trogoderma* pupae treated at different ages could be due to differences in corpus allatum activity at the time of treatment. As precocenes act as destabilizing substrates for oxidative enzymes involved in juvenile hormone biosynthesis (Bowers *et al* 1982); the effects would be directly related to the amount of such enzyme active at the time of treatment. In *Dysdercus cingulatus* Farag and Varjas (1981) related differential precocene susceptibility in different larval instars to instar-dependent changes of corpus allatum activity. However, a direct relationship between corpus allatum activity and precocene sensitivity has not been established. For instance, in *Locusta* following electrocoagulation of the pars inter-cerebralis, juvenile hormone biosynthetic activity of the corpus allatum is drastically reduced, and are as susceptible to precocene as are the fully active glands of the controls (Pratt and Pener 1983). In *O. fasciatus* Masner *et al* (1979) showed that precocene had a stronger inhibitory effect on the less active corpus allatum of young females than on the fully active glands of females one week old yet the apparently inactive glands of last-instar nymphs were insensitive to precocene. In *Diploptera punctata*, precocene is effective both on active and inactive glands (Feyereisen *et al* 1981).

In this study precocene-I has stronger inhibitory effect on pupae of 6, 18, 30, 42, 54 and 90 h old. It is hypothesized that physiological activity of the corpus allatum is a major contributory factor towards precocene susceptibility. The trends in sensitivity to precocene observed in the pupae treated at different ages can be explained by periodic changes in corpus allatum activity that might be related to cycles of reproductive development. Corpus allatum is known to exhibit cycles of juvenile hormone biosynthesis correlated with ovarian development in certain insects (Weaver *et al* 1975). A correlation between juvenile hormone synthesis and oocyte development has been demonstrated in *L. migratoria* (Couillaud *et al* 1984). Adult female *P. americana* normally produces a new ootheca at intervals of 5–6 days and a close relationship between the activity of the corpus allatum and this regular production of oothecae has been demonstrated (Edwards *et al* 1985). The desert locust *Schistocerca gregaria* and the cockroaches *D. punctata* and *Nauphoeta cinerea* (Tobe *et al* 1985; Lanzrein *et al* 1978) also develop oocytes in synchronous waves,

and the corpus allatum exhibit cycles of juvenile hormone biosynthesis which correspond with the cycles of reproductive activity.

Acknowledgements

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Toxic effects of DDT, malathion and mercury on the tissue carbohydrate metabolism of *Sarotherodon mossambicus* (Peters)

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Abstract. Toxic stress due to DDT, malathion and mercury on the tissue carbohydrate metabolism of *Sarotherodon mossambicus* revealed the following manifest effects: (i) concentration of free sugars in the liver and muscle increased due to mobilisation of it from its bound form, glycogen and (ii) the normal carbohydrate metabolic pathway was altered indicating a switch over to anaerobic state involving the conversion of sugars into more lactate via pyruvate.

Keywords. Toxicosis; lactic acidosis; stress syndrome; extracarbohydrate source.

1. Introduction

Studies on blood sugars of fishes exposed to toxicants have demonstrated that tissue carbohydrate sources are mobilised resulting in what is known as hyperglycemia (Holmberg *et al* 1972; Grant and Mehrle 1973; McLeay and Brown 1975; Watson and McKeown 1976). The changes which the free sugars undergo after their mobilisation from bound state remain to be understood. Studies on fishes subjected to physical stress have revealed that lactic acid and pyruvic acid levels are altered following the changes in the tissue carbohydrates (Black 1957; Black and Barrett 1957). The above studies also suggested that lactate and pyruvate concentrations in tissues serve as good indices to assess the stress manifestations. Very little is known about the effects of toxic stress on these intermediary metabolites of carbohydrates. Hence, in the present study, the effects of DDT, malathion and mercury (mercuric chloride) on the blood and tissue carbohydrates and their intermediate metabolic derivatives namely lactic and pyruvic acids were determined.

2. Materials and methods

Specimens of *Sarotherodon mossambicus* were obtained from the fresh water ponds of Tamil Nadu State Fisheries Research Station, Chetpet, Madras. They were maintained in large aquaria ($110 \times 85 \times 75$ cm) for a period of two weeks for acclimation as suggested by Chavin and Young (1970). During the period of acclimation, the fishes were fed with dried prawn powder mixed with cooked rice *ad libitum*. Water was changed on alternate days. Well water was used for both acclimation and experimentation. Water temperature was maintained at $30 \pm 1°C$. From the stock animals maintained as mentioned above, 3 sets, each set comprising 10 individuals were exposed separately to 0·01 ppm DDT, 0·95 ppm malathion and 0·09 ppm of mercury, the respective sublethal concentrations. The pesticides were supplied by Parry and Company, Madras, while the analar grade mercuric chloride

was used as the source of mercury. The sublethal concentrations were deduced by multiplying an application factor of 0·25 to the LC_{50} values of the above compounds.

The fishes used for the above sublethal experiments varied in weight from 15–20 g. Every 24 h, the exposed fishes were transferred into fresh solutions of toxicants. The fishes were fed during the experimental periods. The surviving fishes in each treatment and control were sampled at intervals of 24 h, 7 and 15 days. Before the removal of tissues (liver and muscle) blood was collected from the fishes by tail severance. Potassium oxalate (0·8%) mixed with ammonium oxalate (1·2%) was used as the anticoagulant for rinsing the pipette before withdrawing blood. Blood and tissue lactate were determined by the method of Barker and Summerson (1940). Pyruvic acid was determined following the procedure of Friedemann and Haugen (1943). Tissue glycogen and free sugars were estimated by using the anthrone reagent (Roe 1955; Caroll *et al* 1956). All determinations were carried out in Bausch and Lomb Spectronic 20 Spectrophotometer. The values obtained for each toxicant at the 3 intervals were compared with the corresponding control values by Student's *t* test. The significance was attached to differences at 0·05 level.

3. Results

The results on tissue carbohydrate metabolites are given in table 1. In the liver of DDT exposed fishes 66% depletion of the total initial level was noticed after 15 days. In contrast, the muscle glycogen content showed 90% depletion. The free sugars in liver increased after 24 h and 7 days. In muscle it increased after 24 h and 15 days with a decline in between at 7 days. The liver and muscle glycogen in malathion exposed fishes decreased by 65 and 85% respectively at the end of 15 days. The glycogen in the liver of mercury exposed fishes showed 80% decline while its depletion in muscle was significant even after 24 h. The free sugars in the liver and muscle of these two groups also remained at a higher level corresponding to the glycogen decrease. In the muscle of these groups the free sugars increased at the end of 15 days.

In the liver, significantly higher levels of lactic acid persisted at all the 3 intervals in DDT and malathion exposed fishes while in mercury exposed ones its level increased significantly after 24 h and 7 days but became insignificant after 15 days from that of the control. The pattern of changes in blood lactic acid levels in malathion and mercury exposed groups conformed to its corresponding levels in the muscle at all intervals. On the contrary, in DDT exposed fishes, there was no conformity in the blood and muscle lactate levels, except at 24 h. Tissue pyruvic acid increased following the depletion of glycogen.

4. Discussion

In the control group, the lactic acid in blood and muscle showed differences between the 3 intervals viz., 24 h, 7 and 15 days. The above variations could be due to changes in the normal metabolism of the fishes and also to the smaller blood volume by which the exchange of metabolites from and into the tissues is mediated. Previous studies also reported fluctuations and transitory rise and fall of blood sugars and tissue lactate in normal fishes (Black and Barrett 1957; Chavin and Young 1970).

Table 1. Effects of toxicants on metabolites.

Exposure	Metabolites									
	Glycogen		Free sugars		Pyruvic acid			Lactic acid		
	Liver (μg/100 mg wet wt.)	Muscle (μg/100 mg wet wt.)	Liver (μg/100 mg wet wt.)	Muscle (μg/100 mg wet wt.)	Liver (μg/100 mg wet wt.)	Muscle (μg/100 mg wet wt.)	Blood (μg/ml)	Liver (μg/100 mg wet wt.)	Muscle (μg/100 mg wet wt.)	Blood (μg/ml)
Control										
24 h	5·83±0·72	0·15±0·02	3·41±0·48	0·274±0·023	1·69±0·47	0·82±0·25	12·88±1·43	18·00±8·22	32·32±8·38	22·16±5·55
7 Days	5·32±0·59	0·16±0·022	3·85±0·58	0·274±0·027	2·21±0·30	0·74±0·03	12·93±0·97	17·52±2·61	21·99±2·61	14·75±4·12
15 Days	5·88±1·07	0·18±0·0035	3·12±0·34	0·267±0·025	2·33±0·51	0·62±0·03	12·94±0·93	16·14±8·85	48·04±10·17	22·41±10·37
DDT										
24 h	3·27±0·44*	0·11±0·02*	9·35±0·45*	0·453±0·035*	5·74±1·118*	1·96±0·47*	9·82±0·92*	114·77±11·78*	89·69±17·09*	81·83±30·49*
7 Days	2·78±0·25*	0·026±0·011*	7·48±2·30*	0·208±0·015*	6·73±2·86*	2·03±0·64*	12·08±0·93	71·70±14·31*	163·02±73·68*	32·91±5·74*
15 Days	1·20±0·19*	0·014±0·005*	1·20±0·19*	0·863±0·169*	5·31±1·08*	1·96±0·52*	9·62±1·02*	78·74±20·29*	109·83±10·50*	60·66±9·11*
Melathion										
24 h	3·80±0·93*	0·18±0·02	8·54±2·91*	0·962±0·101*	3·62±0·91*	1·24±0·37	10·77±0·98*	79·57±10·21*	77·17±20·02*	47·08±11·05*
7 Days	2·01±0·67*	0·032±0·016*	16·89±7·64*	0·270±0·015	4·95±1·76*	1·44±0·22*	13·76±0·87	128·56±36·97*	22·30±6·95	19·84±5·35
15 Days	1·27±0·35*	0·028±0·008*	13·31±0·60*	0·561±0·032	4·33±1·52*	1·83±0·40*	11·59±0·86*	42·08±13·96*	50·78±11·45	28·41±3·72
Mercury										
24 h	3·40±0·38*	0·06±0·005*	3·77±0·45	0·404±0·056*	2·52±0·49*	1·22±0·20*	12·03±1·07	7·94±1·84*	70·63±5·47*	15·41±2·53*
7 Days	1·93±0·09*	0·06±0·008*	5·29±1·82	0·171±0·09*	2·26±0·53	0·99±0·37	13·77±0·86	37·92±17·93*	69·46±20·00*	17·25±1·21
15 Days	1·04±0·34*	0·016±0·008*	11·42±3·90*	0·251±0·015	3·41±0·38*	0·84±0·29	11·04±0·46*	13·01±4·84	51·37±4·37	14·66±2·85

*Significant (P = 0·05). Values are given as Mean ± SD.

Hence the levels of metabolites in experimental groups were compared to the corresponding levels in the control fish at the respective time intervals.

Increase of lactic acid in tissues is attributed to the inadequacy of oxygen supply in cells to cope up with complete breakdown of carbohydrates to carbon di-oxide and water (Seskin and Levine 1947). Hence the persistence of tissue lactate in fishes exposed to DDT and malathion in the present study implies hypoxia. Significant decrease in the oxygen consumption of the whole animal and corresponding decrease in the succinic dehydrogenase (SDH) enzyme activity in the liver and muscle of DDT and malathion treated fishes also support the above implication of tissue hypoxia (Ramalingam 1980, 1985).

Moreover the persistence of higher levels of lactic acid in both muscle and liver of DDT group while in the liver of malathion exposed fishes at all time intervals may also be due to impairment in the diffusion of it from the above tissues. Such impairment in the diffusion of tissue lactate into the blood has been reported in stressed fishes. The stasis in the portal vessels has been demonstrated to enhance the above (Heath and Pritchard 1965). The histopathological changes in the liver of DDT and malathion exposed fishes which revealed occlusion of red blood cells in the periportal vessels of liver also indicated heart's congestion and stasis during toxicity in this species (Ramalingam 1985).

In fishes exposed to mercury, both liver and muscle showed no build up of lactate at the end of 15 days. This indicates a mechanism in Hg-exposed fishes for the elimination of lactic acid. The possibility of lactic acid being excreted in the urine of fishes could be inferred from the studies of Karuppannan and Kutty (1978) who have shown that *Tilapia mossambica* (*Sarotherodon mossambicus*) is capable of excreting as much as 25 μg/kg/h of lactic acid under forced swimming stress. In addition to the excretion of lactic acid, evidences are also available which suggest that it could be eliminated through gills (Bates and Vinsonhaler 1956). Studies on anxiety neurosis stress in human also demonstrated that lactic acid accumulates in the tissues, in order to couple with cations such as Ca^{2+}, Mg^{2+} and K^+ (Wallace 1975).

The free sugars levels in the present study reveal that in addition to glycogen other sources such as sugars bound to protein or protein itself may also be involved in the contribution of free sugar pool increase in fishes under toxic stress. The increase in free sugar concentration after 15 days in the muscle of DDT and malathion groups suggests the above. Such extra-carbohydrate sources contributing to free sugars pool have been reported earlier in rodent toxicity studies (Coleman 1968). Significant decline of tissue proteins (liver and muscle) and also the qualitative changes in the electrophoretic fractions of serum proteins in this fish after 7 and 15 days of exposure to the above toxicants (Ramalingam and Ramalingam 1982) also suggest the sparing action of protein reserves to energy demand evoked by toxic stress.

The tissue pyruvic acid increased following the depletion of glycogen. Increase in tissue pyruvic acid in fishes subjected to stress conditions is not uncommon. However, the conversion of pyruvic acid and its further changes either through oxidation or reduction, depends on the expression of the different isomeric forms of lactate dehydrogenase (LDH) enzyme. The changes in the isoenzyme patterns of LDH in serum, liver and muscle of this species (Ramalingam 1985) exposed to DDT and malathion suggest that glycogen that has been solubilised is ultimately forming the fatigue product namely lactic acid.

The sequential changes in the carbohydrate intermediate metabolites suggest the possible mechanisms by which cellular metabolism is affected during toxicity
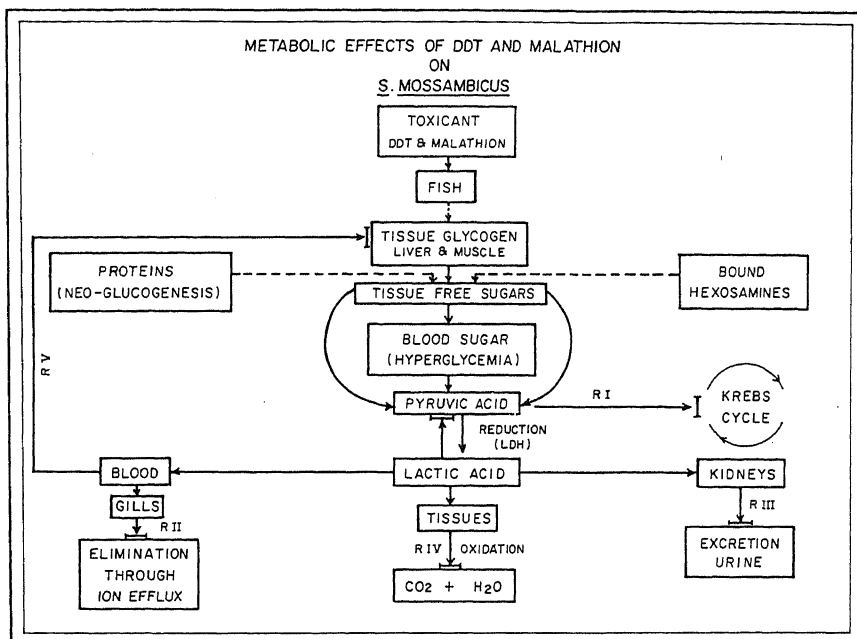
Figure 1.

ure 1). The lethality to fishes by toxicants may be due to the dysfunctioning of 5
erent routes R-I, R-II, R-III, R-IV and R-V through which the intermediate
abolites are either used or eliminated in the normal unstressed fishes. The results
he present investigation, revealing a striking similarity to the features of stress
drome of Selye (1950a, b), could be ascribed to hormonal imbalance.

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'Dark' type Purkinje cells and neuronal ageing

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Abstract. Eighty Wistar rats from age-groups of 3, 12 and 36 months were selected at random from the inbred colony. Ten animals from each group were treated with the ageing-reversal drug, centrophenoxine (100 mg/kg/day, intraperitoneally for 10 weeks). The Purkinje cell population of the brain of these animals were scanned histopathologically to record the impact of chronological age on the rate of conversion of 'light' into 'dark' type. The population of 'dark' cells increased by 52% from 3–36 months. The 'dark' neurons, even in the 3 month-old animals had substantial amount of histochemically detectable and auto-fluorescent age-pigment. While centrophenoxine could remove most of the pigment present in the 'light' type Purkinje neurons it failed to influence the pigment in any way in the 'dark' cells. The drug could not also influence the conversion of the 'light' cells into 'dark' type.

Keywords. Purkinje neurons; cerebellum; ageing; histochemistry.

1. Introduction

The presence of 'dark' cells was first recorded by Flemming (1882) in the spinal ganglion cells. Since then a large number of workers have assigned various reasons to the appearance of 'dark' cells. A few important ones are: postmortem changes (Scharer 1938), asphyxia (Windle *et al* 1944; Rand and Courville 1946), damaging effect of the fixatives (Gerhard *et al* 1948) and regeneration or recovery phase (Bohra and Gupta 1983). The present study reveals a relationship of the appearance of 'dark' cells with the ageing of animals in the cerebellar cortex of Wistar rats—a relationship established never before. The other attempt made was to see if the ageing–reversal drug, centrophenoxine (CPH), can influence the rate of conversion of 'light' type Purkinje cells to 'dark' type, and if CPH can influence the age-pigment in the 'dark' cells.

2. Materials and methods

A total of 80 randomly selected Wistar rats from the inbred colony of the University Animal House were used from 3 age-groups, i.e., 3, 12 and 36 months. A set of 50 animals were sacrificed without any treatment. Ten animals from each age-group were treated with CPH (100 mg/kg/day, intraperitoneally daily) for 70 days and were sacrificed on the 71st day. Cerebella of all these animals were variously fixed and processed for histochemical analysis of the Purkinje cells.

Sagittal and parasagittal sections were studied under the light and fluorescent microscopes. One hundred Purkinje cells in a section were counted to note the percentage of 'dark' cells in the cerebella and 30 sets of cells were scanned in every parameter.

3. Results

Two distinct types of Purkinje neurons were observed in the various histological and histochemical preparations: (i) the normal or 'light' type having a well-marked demarcation between the nuclear and the cytoplasmic regions, and (ii) the 'dark' type staining so intensely in the cytoplasmic as well as nuclear areas that no demarcation could be made out between the two regions.

3.1 *Cytochemistry of 'dark' cells*

The 'dark' cells were present in about all the histological and histochemical preparations (viz., haematoxylin and eosin, toluidine blue, Nile blue sulphate, acid haematin, methyl green pyronin y, periodic acid-Schiff, Zehil-Neelsen test, chromealum haematoxylin and test for acid phosphatase); (figures 1–4) of all the age-group animals studied. In lipid preparations of the gelatin sections, the 'dark' cells were usually of normal shapes. In other preparations, however, they were
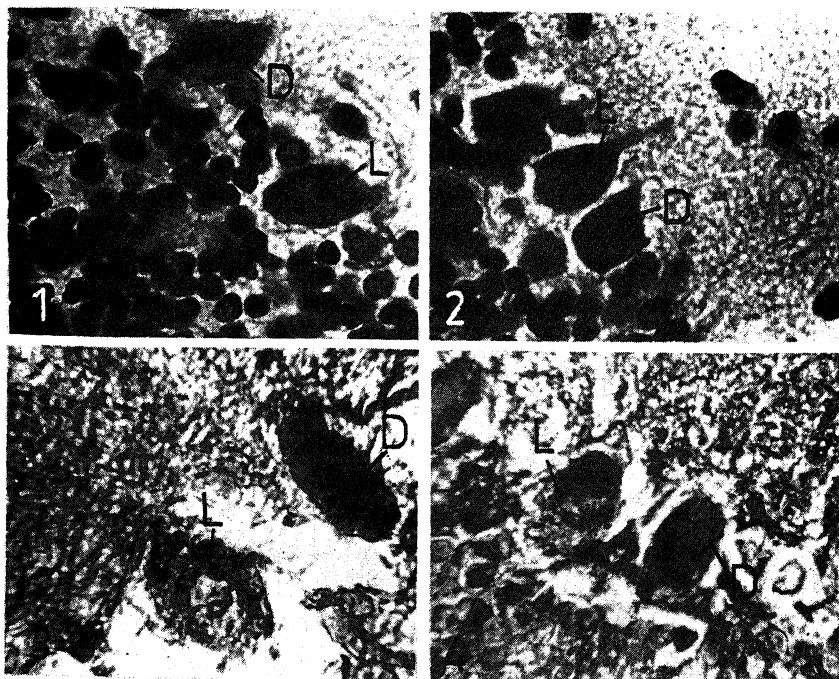
Figures 1–4. **1.** A haematoxylin-eosin preparation from young control group presenting 'light' (L) and 'dark' (D) type Purkinje cells showing a clear difference of both the cell types. A clear nucleolus and nuclear area are seen in L type cells. **2.** A toluidine blue preparation from adult control group depicting the facts as in figure 1. **3.** A Nile blue sulphate preparation of a 10 weeks CPH-treated senile animal showing a heavy deposition of lipofuscin in the 'dark' (D) cell. A reduction in the pigment in the 'light' cells was recorded. **4.** Adult 4 weeks CPH-treated Sudan black B preparation presenting the 'light' (L) type cell with sudanophilic age-pigment at the base of the principal dendrite. The 'dark' (D) cell is coloured homogeneously ($\times 10 \times 100$).

greatly shrunken and possessed somewhat elongated shapes. In the aged animals the 'dark' cells were mostly shrunken in the granular cell-layer.

The 'dark' cells in the cerebella of the young white rats had enough of pigment of autofluorescent nature emitting an orange-yellow refringence when excited with UV light. The normal Purkinje cells had a substantial amount of the age-pigment only after an age of 1 year. Pigment in the 'dark' cells could also be recorded in the periodic acid-Schiff, Zeihl-Neelsen and chromealum haematoxylin preparations and it stained dark red and/or violet with a sharp-yellow-brown refringence in their medullae.

3.2 Quantitative changes with ageing

In cell-counts it was observed that the population of 'dark' cells increased by 52% from 3 months to 3 years. The percentage of 'dark' cells at various age-periods was: 3 months, 16%; 12 months, 28%; 36 months, 43% (table 1, figure 5). There is no appreciable cell-loss in the Purkinje cell-layer with age.

Table 1. Percentage population of 'dark' type Purkinje neurons at various age-periods and after CPH treatment for 10 weeks.

	Young		Adult		Senile	
	Control	Treated	Control	Treated	Control	Treated
'Dark' type Purkinje neurons (percentage of the total population)	x:16	x:17	x:28	x:29	x:43	x:46
	SD:4·38	SD:5·62	SD:8·01	SD:7·2	SD:8·50	SD:10·18
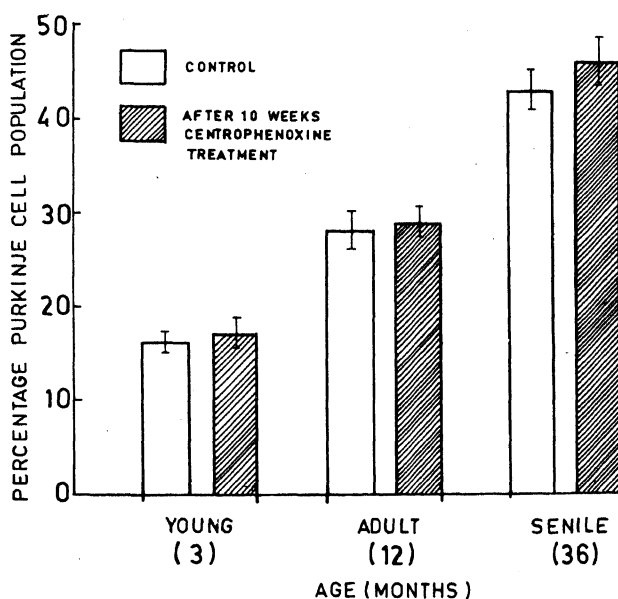
Figure 5. Percentage of Purkinje neurons being transformed into 'dark' type at various age intervals and after centrophenoxine treatment.

3.3 *Influence of centrophenoxine on 'dark' cells*

While CPH could remove most of the pigment present in the 'light' type Purkinj[e]
cells, irrespective of the age of the animal, the drug failed to influence the pigment i[n]
any way possible in the 'dark' cells. The drug could not also influence the warywar[d]
process of conversion of the 'light' cells into 'dark' type as the population of latte[r]
increased even after CPH treatment (table 1; figure 5).

4. Discussion

In the present studies, an increase in the number of 'dark' Purkinje cells with age wa[s]
obseîved and, to support their relationship with age, was the presence of adequat[e]
amount of lipofuscin pigment in these cells in the young animals.

Einarson (1949) interpreted the 'dark' neurons as functionally more activ[e.]
Manocha and Olkowski (1973) showed an increased number of 'dark' cells in th[e]
protein malnourished animals and attributed the phenomenon to dietary protei[n]
deprivation. They further suggested that 'dark' cells might herald certain clinica[l]
symptoms in the experimental animals. Later, Garcia-Segura *et al* (1977) demon[-]
strated the presence of the PAS-positive and PAS-negative spinal ganglion cells i[n]
dogs and postulated that the staining difference in the two might be related t[o]
functional rhythm. Boselova *et al* (1978), by impregnating the Golgi complex an[d]
observing the thiamine pyrophosphatase reaction, attributed the tinctorial dualis[m]
of Purkinje cells to different functional states.

Although, as remarked above, diverse reasons have been adduced in the literatur[e]
as to the appearance of the 'dark' cells, in the present studies, ageing, with th[e]
concomitant presence of lipofuscin, seems responsible for the appearance of thes[e]
cells.

CPH has been established as a lipofuscinolytic agent (Nandy and Bourne 196[6;]
Hasan *et al* 1974; Patro and Sharma 1984; Patro *et al* 1987). No report is availabl[e]
on the influence of CPH on the 'dark' cells, and the present authors, too, could n[ot]
register any appreciable effect of the drug on these cells.

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Reproductive strategies of an egg parasitoid, *Trissolcus* sp. (Hymenoptera: Scelionidae) on two different hosts

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Abstract. Reproductive strategies in relation to host diversity of a scelionid egg parasitoid, *Trissolcus* sp. on *Eupaleopada concinna* Fab. and *Homoeocerus prominulus* (Tagus) are discussed, followed by the life table analysis of the parasitoid, indicating the preponderance of females due to solitary parasitism on the former host and a balanced sex ratio due to superparasitism on the latter.

Keywords. Parasitoid; life table; superparasitism.

1. Introduction

Search for an effective biological control agent against phytophagous insect pests has often focussed on their egg parasitoids and in particular on the species of the family Scelionidae (Brown 1962; Safavi 1968). Inter and intraspecific variation found in parasitoid sex ratios indicates that an understanding of sex allocation is important to study the reproductive strategies of parasitoids. In majority of parasitic Hymenoptera, sex ratios commonly range from a slight male bias to entirely female broods and it was also well known that many parasitoids regulate their sex ratios by selecting different stages of hosts or even different species of host itself (Clausen 1940; Walter 1983). Further, behavioural diversity of adult parasitoids in the process of host location determines the efficiency of a given species as an effective control agent. This paper highlights the unique sequence of behaviour exhibited by females of *Trissolcus* sp. at the time of oviposition and the host specific sex ratio allocation strategy of this parasitoid. Life table studies were also carried out with this parasitoid on the eggs of *Eupaleopada concinna* (Pentatomidae) and *Homoeocerus prominulus* (Coreidae).

2. Materials and methods

The two hosts *E. concinna* and *H. prominulus* were reared in the respective hosts in the laboratory to ensure supply of fresh eggs to the ovipositing female wasps. The former species was collected from *Azadirachta indica* A juss. and *Casuarina equisetifolia* Forst. and the latter from *Prosopis spicigera* L., *Acacia leucophloea* L. and *Cassia marginata* Roxb. Life table analysis of the parasitoid has been carried out on both the hosts following the formula of Birch (1948) and elaborated by Watson (1964) and as presented earlier for two other scelionids by Velayudhan (1987a).

Behavioural observations at the time of oviposition were also made by offering eggs at various stages of development, ranging from freshly laid to those where the host is in an advanced stage of development.

For scanning electron microscopic (SEM) studies of eggs the material was carefully cleaned with a brush, washed repeatedly in alcohol, mounted on to an aluminium

stub using a double adhesive tape, and coated with gold for 3–4 min, using a standard ion coater. The micrographs were than made using a Hitachi table top model S415 A with an emission current of 15 KV.

3. Results

On locating a group of host eggs, the female *Trissolcus* sp. may still not accept it for oviposition, if she perceives that it is unsuitable in some way. This assessment among parasitoid is mainly based on the sensilla of the antennae and ovipositor and especially the latter, since it is considered as a multipurpose organ aiding in the positioning of the egg, supplementary feeding, and to determine the suitability of the egg for oviposition.

3.1 *Ovipositional behaviour*

The behavioural sequence can be split into 4 distinct phases, viz., (i) drumming and turning, (ii) drilling, (iii) probing and (iv) oviposition (figure 1).

After the initial survey and host acceptance, the process of ovipositor insertion was repeated several times, followed by surveillance of the vicinity before oviposition (figure 2, C–D). It takes on an average of 30 ± 8 min for the whole process to be accomplished and after egg laying the female indulged in the process of post-ovipositional preening. SEM studies indicate the absence of any prominent sensilla on the ovipositor. This species was found to be an aggressive individual and usually only one female occupies an egg mass an.· ·y subsequent intruder was chased away. These female parasitoids were ·so observed to feed on the liquid exudate from the
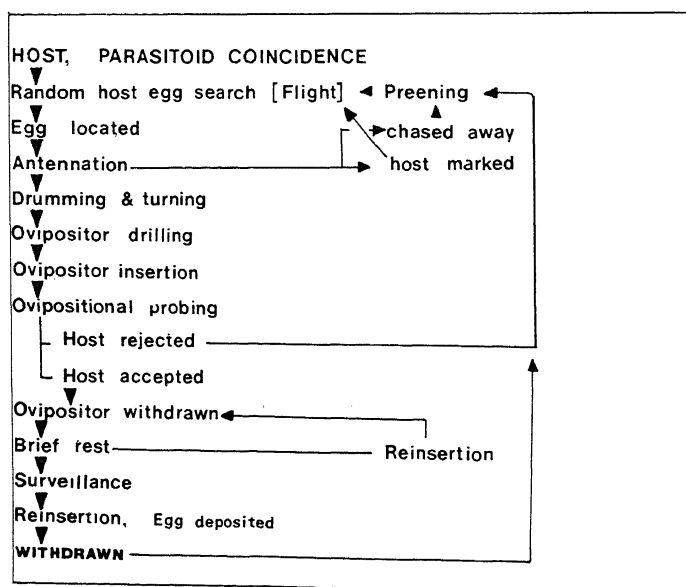
Figure 1. Ovipositional behaviour of *Trissolcus* sp.
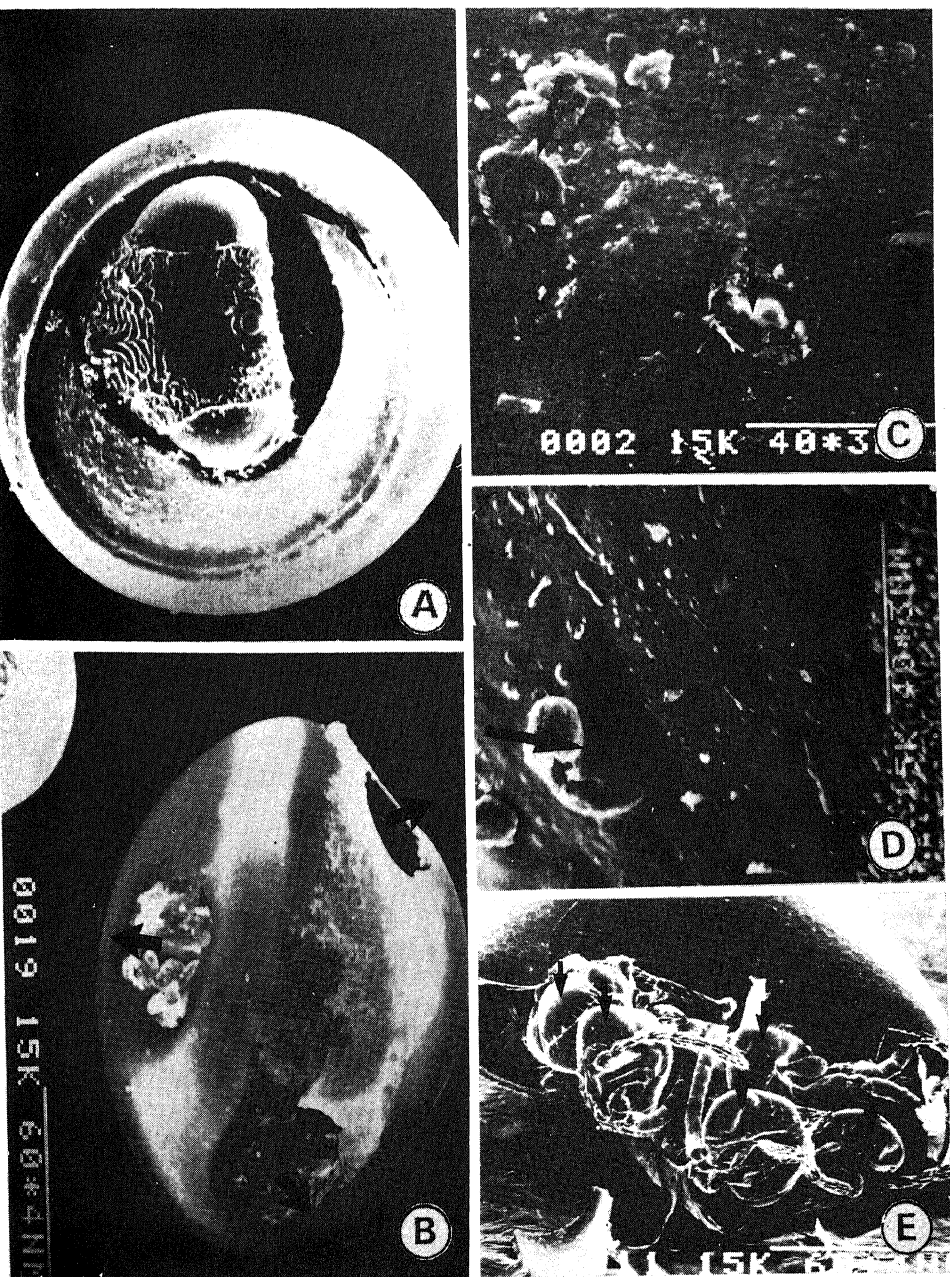
Figure 2. A. Emergence of *Trissolcus* sp. from the egg of *E. concinna*. **B.** Multiple emergence sites of *Trissolcus* sp. on the egg of *H. prominulus*. **C.** Ovipositional punctures of *Trissolcus* sp. on the opercular area of the egg of *E. concinna* (arrow marks). **D.** Ovipositional puncture on non-opercular area of the egg of *E. concinna*. **E.** Multiple embryos in the egg of *H. prominulus* (arrows indicate individual parasitoids).

host egg after the initial ovipositional puncture, presumably to enhance its reproductive potential.

3.2 *Life table studies*

The number of female progeny produced by *Trissolcus* sp. ranged from 40–62 individuals per female and the sex ratio was 1 : 5·3 in favour of the females on the eggs of *E. concinna*. The maximum mean female progeny per day by the parasitoid was attained on the 4th day and the production ceased by the 10 day after the first oviposition (figure 3). The intrinsic rate of natural increase (*rm*) value was 0·2775/female/day and the population multiplied 50 times in a generation time of 14·098 days (table 1).

On the contrary, the life table studies of *Trissolcus* sp. on the eggs of *H. prominulus* showed that the sex ratio was 1 : 1·4 in favour of females and the *rm* value was 0·1665/female/day. The population multiplied 39·9 times in a generation time of 16·86 days (table 1 and figure 4).

3.3 *Superparasitism*

Though *Trissolcus* sp. was considered to be a solitary parasitoid, it showed high incidence of this phenomenon on the eggs of *H. prominulus* only, resulting in the production of a higher number of male individuals. Three to five parasitoid individuals emerged from the superparasitised eggs of the coreid and all the male individuals had
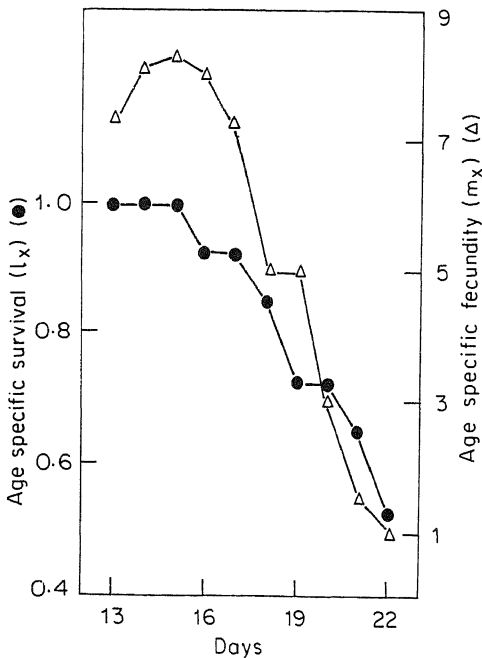
Figure 3. Age specific survival and fecundity of *Trissolcus* sp. on *E. concinna*.

Table 1. Life table statistics of *Trissolcus* sp. on two different hosts.

Parameters	Hosts	
	E. concinna	H. prominulus
Development time (in days)	12·0	11·0
Age at first reproduction (α)	12·0	12·0
Mean number of eggs/female (mx)	56·70	46·60
Net reproductive rate (Ro)	50·00	39·90
Mean generation time (T)	14·098	16·860
Capacity of increase (rc)	0·2860	0·1709
Intrinsic rate of natural increase (rm)	0·2775	0·1665
Cohort generation time (Tc)	13·676	16·83
Finite rate of increase (λ)	1·320	1·180

Figure 4. Age specific survival and fecundity of *Trissolcus* sp. on *H. prominulus*.

reduced body size (figure 2E). Each individual from these eggs made independent outlets on the non-opercular region of the egg during the course of emergence (figure 2B). On the other hand only one individual emerges per egg of the host in the case of *E. concinna* with a preponderance of female individuals (figure 2A).

4. Discussion

The intraspecific variation in the sex allocation of *Trissolcus* sp. suggests that this parasitoid changes sex allocation patterns in different hosts based on variety of

exogenous and endogenous stimuli. In the present investigation host size, shape, texture, curvature and colour appears to be the exogenous factors believed to be utilised by the parasitoid in progeny allocation. Further a parasitoid on locating a host must take 2 decisions—the number of eggs to be laid and sex ratio to be maintained and the latter being under maternal control in these arrhenotokous haplo-diploid hymenopterans (Waage and Ng 1984). The results of life tables clearly indicate the fact that a higher number of females are produced from the eggs of *E. concinna* when parasitised solitarily, while superparasitism of the eggs of *H. prominulus* resulted in a more balanced sex ratio. The super parasitisation and a higher male progeny in the case of the coreid host, appears to be a reproductive adaptation of this parasitoid to utilise less suitable hosts for the production of males. This adaptive nature of differential sex allocation of *Trissolcus* sp. in two different hosts concurs with the findings of Alphen and Thunnissen (1983) on the hyperparasitoid *Pachycrepoideus vindemiae* Rondani. Further short term sex ratio shifts have been observed in the case of the solitary egg parasitoid, *Anastatus ramakrishnae* where males are produced on smaller eggs of pentatomids like *Nezara virudula* and *Acrosternum graminea*, while a high percentage of female progeny was produced on larger eggs of *Halys dentatus* (Velayudhan 1987b).

Occasionally various host eggs are attacked by a single parasitoid due to their accessibility in a particular niche and not due to the order of preference. Above all differential patterns of sex allocation to various hosts depends not only on the host size but also on the nutritional quality (Charnov *et al* 1981; Charnov 1982). Among various chemical profiles, higher lipid and protein content of the host appear important and significantly influence the fecundity of the parasitoids with increased female sex ratio (Muthukrishnan and Senthamizhselvan 1988). Scelionids appear to recognise eggs parasitized by conspecifics and altering their sex ratio in response to the presence of other wasps (Hamilton 1967; Suzuki and Iwasa 1980). There are a number of other circumstances which may constrain a female to lay only male eggs due to such factors as climate (Viktorov 1976) and disease (Zelinskaya 1983).

Thus the results suggest that the female *Trissolcus* sp. often learn the host parameters which ultimately act as an indicator for sex ratio shifts in addition to the nutritional quality, wherein the parasitoid treats the pentatomid egg for female progeny and coreid egg for males and finally providing a basis for parasitoid sex ratio shifts for mass rearing systems.

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Natural food of the edible oyster *Crassostrea madrasensis* (Preston) of Pulicat lake, south India

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Abstract. Fortnightly sampling of the edible oyster, *Crassostrea madrasensis* was made from the natural bed of Pulicat lake during the years 1980–1982. The stomach contents of the oyster composed of 52·8% of diatoms, 45·7% of detritus and 1·5% animal matter. Among phytoplankton, the following is the order of importance: *Navicula, Coscinodiscus, Nitzschia, Pleurosigma, Rhizosolenia, Amphora* and *Peridinium*; and among zooplankton, bivalve veliger ranked first, followed by the ciliate tintinnids. Plankton was also collected from the natural bed to correlate with the gut contents of oyster. The oyster showed preference especially for diatoms like *Pleurosigma, Coscinodiscus* and *Peridinium*, even though these diatoms were found in low quantities in the natural bed area. Two peaks of feeding intensities were observed one during December–January and the other during May–June. Oyster fed poorly during monsoon season (October–November) due to prevalence of low saline conditions in the lake.

Keywords. *Crassostrea madrasensis*; plankton food; diatoms; detritus; zooplankton; feeding intensity.

1. Introduction

Edible oysters are sessile and filter feeders. Generally they feed on the suspended particles and phytoplankters found in the water in which they live. Some of the notable contributions on the food and feeding habits of *Crassostrea madrasensis* are those of Hornell (1908), Moses (1928) and Devanesan and Chacko (1955); of *Ostrea edulis* by Orton (1927); of *Crassostrea virginica* by Jorgenson (1952), Jorgenson and Goldberg (1953), Loosanoff (1949), Loosanoff and Davis (1952), Davis and Guillard (1958), Walne (1958) and Galtsoff (1964) and *C. gryphoides* by Durve (1964). Despite the fact that several workers have attempted to study the food and feeding habits of oysters, there has not been any attempt to study in detail seasonal variations in the intensity of feeding and the selectivity in feeding.

2. Materials and methods

Random fortnightly samples of oysters were collected from the oyster beds of Pulicat lake, for two years from July 1980 to June 1982. The oysters were preserved in 5% formalin immediately. In the subsequent week, after taking morphometric measurements, the oysters were shucked and the shells removed. The condition of the gonad was noted by the smear method under a microscope. A narrow mouthed pipette was slowly introduced through the mouth till it reached the stomach and the gut contents were pipetted out and placed in the plankton counting chamber. This was repeated for a minimum of 5 times to remove as much of the contents as possible.

Unlike fishes, the quantity of food consumed by oysters is very little, which makes it difficult to determine the actual volume by the displacement method. Therefore the points method of Hynes (1950) for fishes was applied in the present study. Points were allotted i.e., 100, 75, 50, 25 and 10 for the quantity of matter removed from the gut of oysters. The contents of the gut were spread well in the plankton counting chamber and the number of diatoms, dinoflagellates and zooplankters were counted. All detritus and unidentifiable algal matter were grouped together and were expressed separately in percentages. The final analysis of the food of *C. madrasensis* was made by the method of index of preponderance of Natarajan and Jhingran (1961) using occurrence and numerical abundance of each food item.

Samples of plankton were also collected from the oyster bed by filtering 200 litres of water through a hand-net made of fine mesh bolting silk. The plankton was preserved in 2% formalin and analysed subsequently. The plankton concentrate was made up to 100 ml in a measuring cylinder. After thoroughly mixing the contents 1 ml was transferred to the plankton counting chamber and the various phyto and zooplankton were identified up to the generic level and counted under a compound microscope. The same was repeated 5 times and the average was taken into consideration and expressed as numbers per 100 litres of water. The environmental parameters such as salinity, temperature and dissolved oxygen were also recorded regularly from the oyster bed.

3.   Results

3.1   *Natural food of oysters*

The stomach contents of oysters in the Pulicat lake were constituted by 52·8% of diatoms, 45·7% of detritus and 1·5% animal matter. Monthly variations in the percentage composition of diatoms, detritus and zooplankton from the gut are given in table 1.

3.1a   *Detritus*: The percentage of detritus, unidentifiable plant matter and nannoplankton observed in the gut were grouped together and estimated to be 45·7%. The organic and inorganic detritus were perhaps consumed from the water column, with the opening of the valves of the oysters frequently. Higher percentage of detritus was found in August and February during both the years. The quantity of detritus was very low prior to monsoon as well as during the summer months.

3.1b   *Diatoms and dinoflagellates*: The bulk of the gut contents was constituted by the diatoms and dinoflagellates. Among these food items, the order of preference was *Navicula, Coscinodiscus, Nitzschia, Pleurosigma, Rhizosolenia, Amphora* and *Peridinium*. However, the other plankters, such as *Prorocentrum, Bacteriastrum, Detonula, Cymbella, Rabdonella, Diploneis, Chaetoceros, Thalassiosira* and *Skeletonema* also occurred in the gut, but their occurrence was seasonal. *Noctiluca* and *Ceratium* were also noticed occasionally in the stomach.

3.1c   *Zooplankton*: Among the zooplankters, the bivalve veligers ranked first, followed by the ciliate tintinnids. The occurrence of copepods and polychaete larvae

Table 1. Monthly variations in the percentage composition of detritus, diatoms and zooplankton observed in the gut of oysters.

Month	Year	Detritus (%)	Diatoms (%)	Zooplankton (%)
July	1980	41·25	57·04	1·71
August		48·75	50·47	0·78
September		36·67	63·33	—
October		39·67	60·01	0·32
November		47·50	51·51	0·99
December		44·00	55·58	0·42
January	1981	47·58	48·52	3·90
February		62·75	36·69	0·56
March		44·33	55·22	0·45
April		36·67	60·36	2·97
May		42·50	53·41	4·09
June		28·00	70·57	1·43
Average		43·31	55·23	1·47
July		40·00	59·97	0·03
August		64·56	34·74	0·70
September		47·50	51·79	0·71
October		47·81	51·02	1·17
November		53·90	45·48	0·62
December		47·50	52·18	0·32
January	1982	45·42	53·95	0·63
February		54·50	41·23	4·27
March		54·17	44·14	1·69
April		48·25	48·79	2·96
May		41·56	53·89	4·55
June		32·00	67·35	0·65
Average		48·09	50·38	1·53
Average for 2 years		45·70	52·80	1·50

Each value represents an average estimate of oyster sample containing 20–41 individuals.

was noticed occasionally. The index of relative abundance of different food items has shown (table 2) the maximum occurrence of veliger larvae during November and May. The peak occurrence of tintinnids was observed in April and May during the two years of study.

3.2 Seasonal variations in the food of oysters

The important diatoms which formed the food of the oysters and their fluctuations both in the environment as well as in the gut are illustrated in figure 1. The major food items, *Navicula, Coscinodiscus, Nitzschia, Pleurosigma, Rhizosolenia, Amphora* and *Peridinium* made their occurrence throughout the period of this study with fluctuations. The peak occurrence of these diatoms in the environment coincided with their peak in the gut. However, the oyster showed selectivity in feeding during certain seasons of the observation period. For example, the diatom *Pleurosigma* was found more than any other diatom in the gut during March 1982, but at the same time in the lake water it was found even fewer than the *Rhizosolenia* and *Nitzschia*. Avoiding these two, the oysters preferred *Pleurosigma* during this period. The oysters

Table 2. Index of relative importance of different food items.

Month	Number of oysters	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)
July (1980)	24	1607	2207	569	313	—	1286	813	—	—	631	—	—	—	304	278	80	365	—	—	—	—	—	133	—	—	—
Aug.	27	2545	1963	653	619	—	947	475	—	—	—	—	—	170	58	—	—	655	—	—	—	—	—	140	—	—	—
Sep.	30	2907	2012	634	623	168	195	383	117	—	221	106	—	85	210	473	291	125	—	—	—	—	50	—	—	—	—
Oct.	35	2027	3160	529	422	—	206	240	—	—	—	—	—	—	286	—	—	39	39	—	—	—	—	—	—	—	—
Nov.	20	2766	1729	2184	235	—	564	—	—	—	89	—	129	—	—	—	—	—	—	—	—	—	183	—	—	—	—
Dec.	30	2726	3187	515	274	30	110	348	—	—	—	13	—	—	9	18	61	—	56	—	—	—	75	—	—	—	—
Jan. (1981)	30	1698	1228	572	384	142	410	483	—	—	—	—	—	—	—	98	—	—	—	4	—	—	11	420	—	—	—
Feb.	30	2906	1457	570	1168	257	421	749	—	—	—	—	—	—	88	154	60	26	60	—	—	—	89	38	—	—	—
Mar.	30	2569	2244	919	385	228	470	469	—	—	—	—	—	—	—	73	—	—	173	—	—	—	—	77	—	—	—
Apr.	25	935	4326	344	—	—	230	223	—	268	—	—	—	—	—	—	—	—	—	—	—	—	—	425	—	—	—
May	25	1129	2926	—	509	237	176	1243	—	—	137	—	225	—	—	—	—	—	—	—	909	—	75	578	—	36	—
June	30	1353	1154	1975	85	2220	268	598	42	—	333	16	126	—	—	—	54	—	—	64	6	—	34	25	—	—	—
July	36	2241	835	2464	181	416	500	169	5	—	858	43	—	—	113	—	47	—	28	—	—	—	3	—	—	—	—
Aug.	26	1861	1580	2483	149	40	753	509	103	205	44	—	—	—	—	—	—	21	—	21	93	—	156	—	21	—	87
Sep.	20	2335	752	2617	124	938	478	218	66	—	876	152	118	—	139	—	86	—	—	—	—	—	211	—	—	—	—
Oct.	32	1857	1575	2198	146	595	547	54	—	—	133	—	247	—	67	—	150	—	—	—	—	—	121	—	—	—	—
Nov.	33	1541	1062	1692	77	—	632	—	—	—	—	—	—	—	—	—	215	—	—	—	—	—	—	—	—	—	20
Dec.	24	2259	1154	1776	456	245	199	117	—	—	—	49	136	—	—	—	101	—	—	—	—	—	37	—	—	—	—
Jan. (1982)	30	4169	402	812	139	—	60	366	—	—	435	—	—	—	—	359	—	—	—	—	—	—	112	—	—	—	—
Feb.	20	2345	1627	1195	493	—	370	218	—	—	—	—	—	—	—	265	215	—	—	—	—	—	149	—	—	—	—
Mar.	32	1346	2031	766	213	114	183	2175	—	—	44	24	57	161	—	—	41	—	—	—	—	—	74	269	—	—	—
Apr.	41	1798	2414	—	—	—	131	1772	—	—	—	—	—	—	—	—	—	—	—	—	388	88	6	134	352	—	—
May	32	1022	384	3066	—	225	194	1021	—	259	—	—	233	—	—	—	—	—	—	—	882	—	127	592	—	9	—
June	20	1691	843	1739	145	464	300	689	—	—	—	—	143	484	—	—	—	—	—	—	590	—	92	—	—	—	—

(1), *Navicula*; (2), *Coscinodiscus*; (3), *Nitzschia*; (4), *Amphora*; (5), *Peridinium*; (6), *Rhizosolenia*; (7), *Pleurosigma*; (8), *Thalassiosira*; (9), *Chaetoceros*; (10), *Prorocentrum*; (11), *Skeletonema*; (12), *Biddulphia*; (13), *Proplectella*; (14), *Cymbella*; (15), *Detonula*; (16), *Rhabdonella*; (17), *Bacteriastrum*; (18), *Diploneis*; (19), *Ceratium*; (20), *Bacillariales*; (21), *Noctiluca*; (22), *Velieger*; (23), *Tintinnopsis*; (24), Copepods; (25), eggs; (26), Polychaete larvae.

Figure 1. Occurrence of the dominant diatoms in the stomach contents of oysters, *C. madrasensis* in the Pulicat lake, during July 1980–June 1982.

showed preference for *Coscinodiscus* by taking the less concentrated *Coscinodiscus* from the water and avoiding the highly concentrated *Rhizosolenia* during April 1981. The occurrence of *Peridinium* in the plankton was lower than *Nitzschia* and *Coscinodiscus* during May and June 1981. In the food of the oysters *Peridinium* was found to be dominant.

The other important food items of oysters were *Biddulphia, Bacteriastrum,* veliger

and *Tintinnopsis*. The percentage occurrence of these diatoms and zooplankton in the gut was directly proportional to their occurrence in the environment. *Tintinnopsis* was found to be maximum in the month of May during the two years of observations. Bivalve viligers showed their peak during May and October–November whenever there was spawning of oysters in the lake.

The stray occurrence of copepods, polychaete larvae, crustacean appendages, eggs, *Ceratium* and *Noctiluca* was also noted in the oyster guts. Their occurrence in the gut may be due to their accidental entry along with other food items.

3.3 *Feeding intensity*

Based on the observations made, two peaks of feeding intensities were noted, one during December and January, and the other during May and June (figure 2). The high feeding during December and January was mainly due to the greater abundance of diatoms such as *Navicula*, *Coscinodiscus* and *Pleurosigma*. Poor feeding was observed during October–November. This was due to freshwater influx into the lake. Oysters close the valves tightly to overcome the unfavourable conditions during this period.
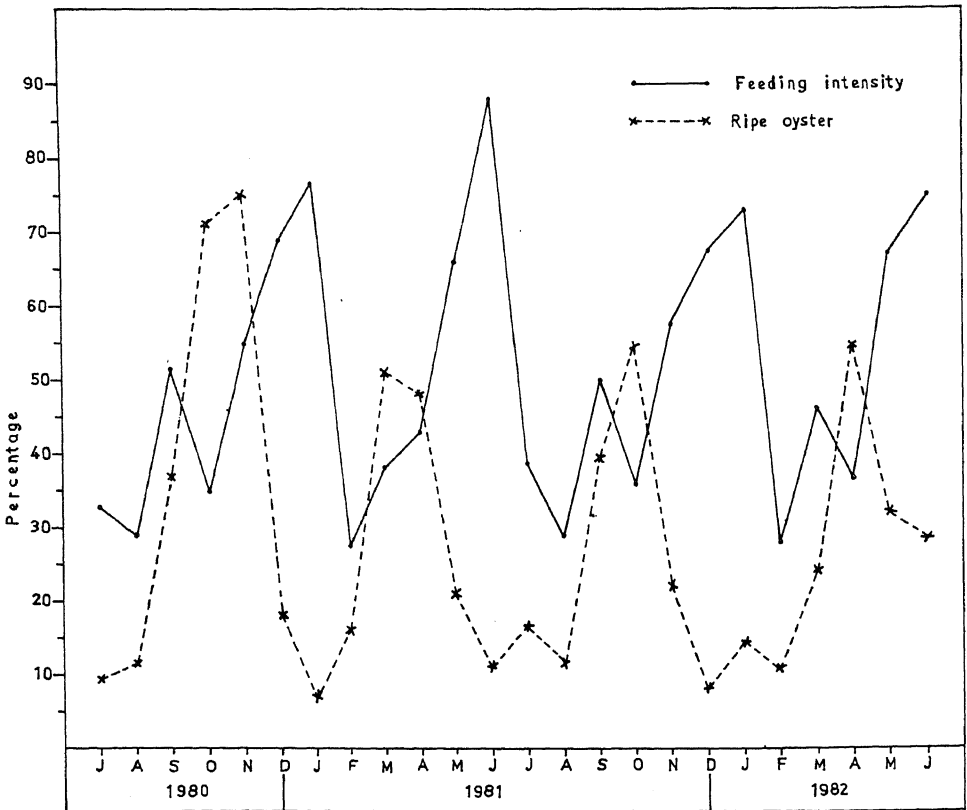
Figure 2. Relative percentage of the feeding intensity and the occurrence of ripe oysters, *C. madrasensis* in the Pulicat lake.

Though the diatom food items are available during the monsoon, silting was also ry high which is unfavourable to the oysters and there was avoiding of food during is period. The feeding intensity was less in February–March and August during the o years of observation. During February–March the oyster bed was exposed ring most of the time and the diatoms were very poor in the environment. The or feeding in August may be due to the low occurrence of diatoms due to evalence of higher salinity and temperature.

The feeding intensity was very high during active gametogenesis and the energy quired for formation of gametes was obtained from the dominant food items. hen the majority of oysters attain the ripe condition, the feeding intensity was und to be very less. Immediately after spawning was over the feeding intensity was und to attain a peak level in December–January and May. This high feeding was ainly to meet the energy lost in connection with the gamete formation and leasing of gametes during the spawning season.

Discussion

ysters which are sedentary in nature, living in the mouth of the estuaries, ckwaters and lagoons face complex of environmental factors with changes in the gimes of salinity and temperature and fluctuations of food from season to season.

the tropical countries, since light and temperature are relatively constant roughout the year, salinity among other factors, is more important in influencing e abundance of food and feeding intensity of oysters. In the Pulicat lake, salinity ays an important role in producing planktonic peak during the summer months. uayle (1980) has mentioned that little is known about the food of oysters. The main bjects such as food preference and utilisation, dispersion of larvae, and aggregtion spat and genetics are poorly known. The present study fills the lacunae in the field food and feeding and food preference by the oysters in Pulicat lake.

Among the food of oysters of Pulicat lake, the high percentage of detritus in the it of oysters suggests its importance as an item of food. The same has been reported Hoek (1883) in the European waters, Blegvad (1914) in the Danish waters, Moses 928) at Madras and Durve (1964) in the Bombay oyster *C. gryphoides*. In the ilicat lake oysters, the percentage of detritus was found to be 45·7% which showed ictuations during the period of this study.

The order of food preference by the oysters may vary from place to place pending upon the environmental conditions. Orton (1937) studied the food of sters and found that the main diatoms on which oysters feed are *Nitzchiella parva*, *eurosigma*, *Coscinodiscus*, *Chaetoceros*, *Rhizosolenia*, *Melosira* and *Prorocentrum* d among protozoans *Tintinnopsis*. Devanesan and Chacko (1955) studied the food oyster *C. madrasensis* and listed diatoms such as *Rhizosolenia*, *Coscinodiscus*, *haetoceros*, *Bacillariales*, *Biddulphia*, *Nitzschia*, *Pleurosigma* and *Guinardia*. Durve 964) observed the major food items to be *Coscinodiscus*, *Thalassiosira*, *Biddulphia*, *oconeis*, *Achnanths*, *Diploneis* and *Synedra* in the oyster *C. gryphoies* from the ombay coast. In the Pulicat lake oysters, the order of preference was *Navicula*, *oscinodiscus*, *Nitzschia*, *Pleurosigma*, *Rhizosolenia*, *Amphora* and *Peridinium* and the otozoan *Tintinnopsis*.

During the monsoon, detritus or silt was at a high level and planktonic production as low as a result of low saline conditions in the lake and hence feeding was also

poor in the oysters. Similar observations were made by Durve (1964) in *C. gryphoides* during the monsoon season. The presence of protozoan and metazoan animals and their remains were noticed in the gut of oysters by Lotsy (1893) and Korringa (1952). In the Pulicat lake, the animal matter constituted 1·5% consisting of mainly veligers and *Tintinnopsis*.

From the present study, it is clear that oysters feed on diatoms and detritus. The major food component being diatoms which is responsible for storage of glycogen in the hepatopancreas during the high feeding intensity and it is supplied to the gonad at the time of gamete formation. The reproductive status of the oysters is mainly determined by their feeding intensity.

Acknowledgement

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Inversion polymorphism in natural populations of *Drosophila nasuta nasuta*

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Abstract. Six south Indian populations of *Drosophila nasuta nasuta* were analysed for inversion polymorphism. A total of 28 inversions were recorded. Of these, 9 are entirely new gene arrangements reported here for the first time. Various facets of the polymorphic system of these 6 populations, namely, flexibility, heteroselection, non-random distribution and linkage disequilibrium are discussed.

Keywords. *Drosophila nasuta*; inversions.

1. Introduction

Drosophila nasuta nasuta, a widespread species, presents a high order structural variability due to inversions in its natural populations. It stands one among the chromosomally most variable species in the genus and tops the list in the *nasuta* subgroup (Nirmala and Krishnamurthy 1974; Ranganath and Krishnamurthy 1975; Rajasekarasetty *et al* 1979). A survey of 6 south Indian populations was made to unravel their polymorphic organization and the findings are presented.

2. Materials and methods

Population samples of *D. n. nasuta* were obtained from 6 places, namely, Gangothri, Sanitorium, Mandakalli, Biligirirangana hills I and II and Kotagiri. Flies were trapped using fermenting banana seeded with yeast as the bait. Egg sample technique (Strickberger and Wills 1966) was employed to analyse the chromosomal constitution. Salivary gland chromosome preparations were made using lacto-acetoorcein stain. The nomenclature of different inversions is after Nirmala and Krishnamurthy (1974) and Ranganath and Krishnamurthy (1975).

3. Results and discussion

In *Drosophila*, the absence of crossing over in males and selective elimination of dicentric and acentric chromatids during meiosis in females, created the possibility for the group to use inversion polymorphisms in structuring of their populations (Freire Maia 1961). Earlier studies of Nirmala and Krishnamurthy (1974), Ranganath and Krishnamurthy (1975) and Rajasekarasetty *et al* (1979) have revealed a total of 46 different inverted gene arrangements in different populations of *D. n. nasuta*. The present analysis of 6 populations of *D. n. nasuta* has exposed a

total of 28 different gene orders. A critical comparison of these rearrangements with those of the already described ones has shown that 19 of these correspond to the ones already recognized while 9 sequences are new. These chromosomal patterns, which are maiden reports, are shown in figure 1. By considering the present and earlier reports, the genetic system of *D. n. nasuta* is found to be highly variable with a tally of 55 inversions. Of these, 3 are located in chromosome X while 4 and 7 inversions are in the left and right arms of chromosome 2 respectively. The remaining 41 inversions are localized in chromosome 3. Eventhough there are inversions in all chromosomes, there is a non-random distribution of inversions in the chromosomes of *D. n. nasuta* with a heavy concentration of inversion breakpoints in the basal region of the chromosome 3. *D. pseudoobscura, D. persimilis, D. nebulosa* and *D. busckii* also present a situation with one of its chromosomes harbouring almost all inversions; while in *D. willistoni, D. robusta, D. algonquin, D. sturtevanti* and *D. paramelanica* polymorphism is distributed in all chromosomes (cf. Dobzhansky 1970; Ranganath 1975). Inspite of the explanations offered by Novitski (1946), Wasserman (1963), White (1973) and Ranganath and Krishnamurthy (1978), the pattern of distribution of inversions among different chromosomes is an intriguing problem that is yet to be understood adequately.

The polymorphic system of the 6 populations of *D. n. nasuta* under study is not the same. There exist both qualitative and quantitative differences among populations (tables 1–5). The Sanitorium population was most heterogeneous with 20 different inversions while the Kotagiri population had just 10 inversions. The mean number of inversions per individual varied from 2·06 of Gangothri population to 1·20 of Kotagiri population. Of the 28 inversions recorded in the present study, only 3A, 3C, 3E, 3E + 3H, 3H + 3K, 2RA and 2RB were found in all the 6 populations. Of these, except 3A and 3C, the frequencies of other inversions differed significantly ($P < 0.05$; table 6). By judging their frequencies, it can be assumed that certain elements of the polymorphic system in *D. n. nasuta* appear to be rigid while others are flexible to the different population-environmental situations.

In the 6 populations of *D. n. nasuta* under investigation, the incidence of individuals with one or more heterozygous inversions was always significantly more than those without any heterozygous inversion. In every population, the frequency of such heterokaryotypes was always more than 50% (table 1). Further, there was no significant difference in their frequencies among different populations (table 5). Thus, heterokaryotypy has become a part and parcel of the population structure of *D. n. nasuta* and this fits into the concept of obligate heteroselection proposed by Ranganath and Krishnamurthy (1974).

Of the 28 inversions observed in the present study, 5 were in chromosome 2 and 23 were in chromosome 3. Frequencies of individuals with inversions in only one of these chromosomes (linked) and inversions in both of these chromosomes (unlinked) in different populations are presented in table 6. It was seen that the number of individuals with only linked inversions was significantly higher than those with unlinked inversions. Possible factors which favour the dominance of individuals with linked inversions over unlinked inversions have been discussed by Anderson *et al* (1967), Copper *et al* (1955), Riles (1965) and Ranganath and Krishnamurthy (1978).

Yet another interesting feature of the polymorphic system of *D. n. nasuta* is the nature of relationship between two independent inversions namely 3A and 3C. In
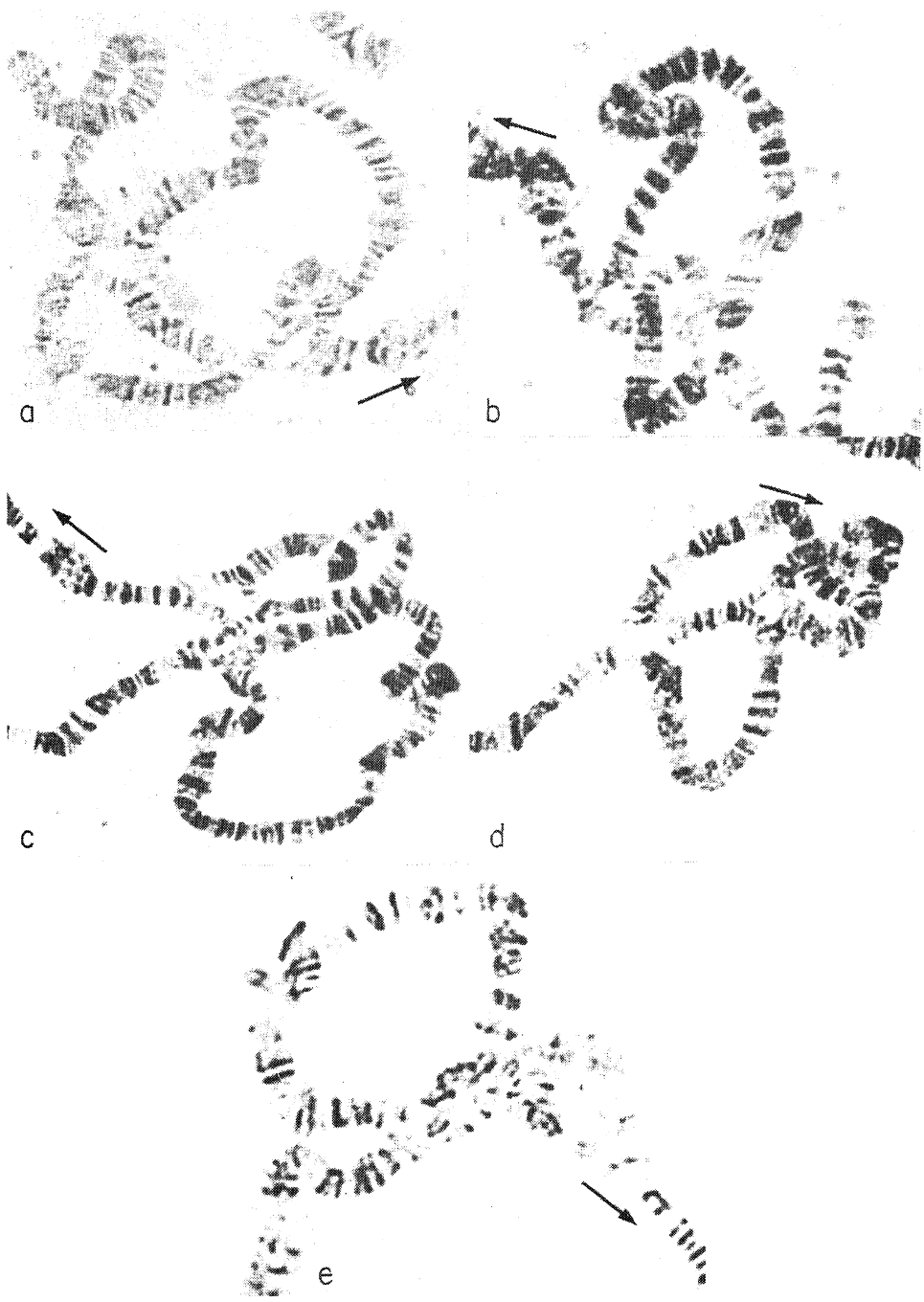
Figure 1. For caption, see next page.
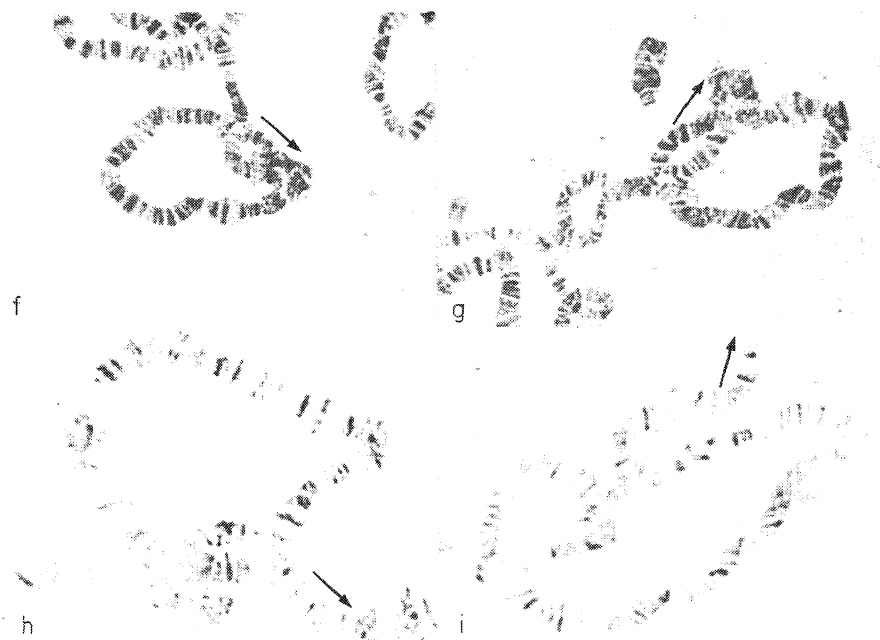
Figure 1a–i. New inverted gene arrangements in the chromosome III of *D. n. nasuta*. Arrows indicate the chromocenter end.

Table 1. Differences in number of inversions, mean number of inversions and heterokaryotypes in 6 south Indian populations of *D. n. nasuta*.

Populations	No. of larvae scored	No. of inversions	Mean No. of inversions per larva	Hetero-zygosity (%)
Gangothri	236	15	2.06 ± 0.08	85.59
Sanitorium	271	20	1.83 ± 0.07	79.70
Mandakalli	289	18	1.65 ± 0.06	77.50
BR hills I	218	14	1.52 ± 0.07	71.10
BR hills II	129	12	1.44 ± 0.10	65.89
Kotagiri	234	10	1.20 ± 0.06	63.24

Table 2. Frequency (in per cent) distribution of II chromosome gene arrangements in 6 south Indian populations of *D. n. nasuta*.

Populations inversions	Gangothri	Sanitorium	Mandakalli	BR hills I	BR hills II	Kotagiri
2RA	9.74	13.28	4.84	0.45	2.32	0.85
2RB	8.05	7.74	8.30	2.29	—	1.28
2RC	—	2.58	2.76	—	0.77	—
2RD	4.66	0.36	1.73	—	—	—
2RG	—	0.36	—	—	—	—
2RA + 2RB	2.11	—	1.73	2.29	0.77	0.85

Table 3. Frequency (in per cent) distribution of III chromosome gene arrangements in 6 south Indian populations of *D. n. nasuta*.

Populations inversions	Gangothri	Sanitorium	Mandakalli	BR hills I	BR hills II	Kotagiri
3A	19·91	14·39	17·99	12·84	18·62	9·82
3C	19·06	14·02	18·33	12·84	18·62	9·82
3E	12·71	20·29	20·06	13·30	22·48	11·96
3H	2·54	—	1·03	1·37	4·65	4·70
3O	—	0·36	1·03	0·91	0·77	—
3V	—	—	—	0·45	—	—
3E + 3H	31·35	27·67	23·87	33·94	17·05	24·78
3H + 3K	16·10	8·11	7·61	8·71	10·07	6·83
3E + 3N	1·69	1·00	1·38	—	—	—
3E + 3J	—	—	—	4·12	2·32	5·98
3O + 3P	0·42	2·58	1·73	0·45	2·32	2·56
3O + 3N	—	0·36	—	—	—	—
3W + 3X	5·50	4·42	3·80	2·29	—	—
3Y + 3Z	—	—	1·38	—	—	—

Table 4. Frequency (in per cent) distribution of the new gene arrangements of III chromosome in 6 populations of *D. n. nasuta*.

Populations inversions	Gangothri	Sanitorium	Mandakalli	BR hills I	BR hills II	Kotagiri
Complex						
I	2·54	—	—	—	—	—
II	—	1·47	—	—	—	—
III	—	1·84	—	—	—	—
IV	—	—	1·03	—	—	—
V	—	—	—	1·37	—	—
VI	—	1·84	—	—	—	—
VII	—	1·47	—	—	—	—
VIII	2·54	0·36	1·03	—	—	—
IX	—	—	—	—	3·87	—

Table 5. Summary of Chi square test computed to compare the frequencies of common inversions and the heterozygosity in 6 south Indian populations of *D. n. nasuta*.

Populations	No. of larvae scored	3A	3C	3E	3E+3H	3H+3K	2RA	2RB	No. of inversion heterozygotes
Gangothri	236	47	45	30	74	38	23	19	202
Sanitorium	271	39	38	55	75	22	36	21	216
Mandakalli	289	52	53	58	69	22	14	24	224
BR hills I	218	28	28	29	74	19	1	5	155
BR hills II	129	24	24	29	22	13	3	0	85
Kotagiri	234	23	23	28	58	16	2	3	148
	ΣX^2	11·00	10·53	13·56	11·75	14·45	56·85	29·41	10·74
df 5;	*P* Value	>0·05	>0·05	<0·05	<0·05	<0·05	<0·01	<0·01	>0·05

all the 6 populations, the number of double heterozygotes for both 3A and 3C was significantly higher than the number of single heterozygotes for either 3A or 3C

Table 6. Frequencies (in per cent) of linked and unlinked heterozygous inversions in II and III chromosomes encountered in the natural populations of *D. n. nasuta*.

Populations	II Chromosome	III Chromosome	II + III Chromosome
Gangothri	6·35	61·44	17·79
Sanitorium	5·53	17·34	9·22
Mandakalli	6·92	69·89	11·41
BR hills I	1·37	22·47	3·66
BR hills II	0·77	61·24	3·10
Kotagiri	2·13	16·66	0·85

Table 7. Linkage disequilibrium between 3A and 3C heterozygous inversions in 6 south Indian populations of *D. n. nasuta*.

Populations	No. of larvae	3A	3C	3A, 3C	ΣX^2	df. 1 P value
Gangothri	236	3	1	44	73·50	<0·001
Sanitorium	271	1	0	38	72·10	<0·001
Mandakalli	289	2	3	50	82·05	<0·001
BR hills I	218	0	0	28	56·02	<0·001
BR hills II	129	0	0	24	48·00	<0·001
Kotagiri	234	0	0	23	46·05	<0·001

(table 7). Out of 1377 larvae only 10 were single heterozygotes (0·72%) while 207 were double heterozygotes (15·03%). This is a clear case of linkage disequilibrium between two linked gene orders. Eventhough many species of *Drosophila* are known to have inversion polymorphism, the phenomenon of linkage disequilibrium is not frequent. It has been recorded only in a few instances (Blight 1955; Levitan and Salzano 1959; Brncic 1961; Mather 1963; Kumar and Gupta 1986).

Thus the polymorphic system of *D. n. nasuta*, featuring a wealth of inversions, flexibility, heteroselection, non-random distribution and linkage disequilibrium is unique among the members of *Drosophila*.

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Cytology and seasonal changes of the pituitary of the emballonurid bat, *Taphozous melanopogon* (Temminck)

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Abstract. The pituitary glands of *Taphozous melanopogon* were examined histologically and cytochemically by employing several cytochemical staining techniques. The neural lobe is composed mostly of nerve fibres amidst which occur numerous small glial cells and a few Herring's bodies. Two cell types have been identified in the pars intermedia. In addition to chromophobes, 5 different cell types, namely, 3 types of basophils (thyroid stimulating hormone, follicle stimulating hormone and luteinising hormone producing cells) and two types of acidophils (lactotrophic hormone and somatotrophic hormone producing cells), were identified in the pars distalis. The gonadotrophs and the lactotrophs aggregate in patches in certain regions of the pars distalis and exhibit cyclical changes in structure and number. The physiological significance of the cyclical changes of these cells is discussed in relation to the reproductive cycle of the animal.

Keywords. Pituitary; seasonal change; *Taphozous melanopogon*; Chiroptera.

1. Introduction

Pituitary cell types and their seasonal changes in relation to the reproductive cycles of bats are of considerable interest because of the diverse reproductive strategies adopted by these animals. Among the more than 900 species incorporated in 17 currently recognised extant families of Chiroptera (Koopman 1984), the structure of the pituitary is known of 4 pteropid (Hanstrom 1950; Bhalchandra 1980, 1985; Badwaik 1986), one rhinopomatid (Karim and Khan 1985), two rhinolophid (Herlant 1953), 4 hipposiderid (Patil 1974; Bhalchandra 1976), one phyllostomid (Richardson 1978, 1979) and a few vespertilionid (Guthrie 1935; Sawyer 1936; Hanstrom 1950; Siegel 1955; Herlant 1956, 1962; Pyere and Herlant 1963; Kobayashi 1966; Azzali 1971) species. Cyclical cytological changes in the pituitary in relation to the sex-cycle in bats have been studied mostly in hibernating vespertilionids (Herlant 1953, 1963, 1964, 1967; Siegel 1955). Among the non-hibernating bats details of the cytology and cyclical changes in the adenohypophysis have been reported in *Miniopterus schreibersii* (Pyere and Herlant 1963), 4 hipposiderids (Patil 1974) and one phyllostomid (Richardson 1978, 1979) species. Since no information is available on the pituitary of any emballonurid bat, a detailed study of this organ in *Taphozous melanopogon* has been undertaken. The anatomy of the pituitary of this species has already been reported (Badwaik 1988). The present study includes observations on the histology of this organ and the seasonal changes in the gonadotrophs and the lactotrophs in the adult adenohypophysis of this bat.

2. Materials and methods

Specimens of *T. melanopogon* were collected at frequent intervals during two

successive years (1983–1984) from dungeons of old forts at Burhanpur and Asirgarh in south-western Madhya Pradesh during all the months except October and November. Altogether 834 specimens were collected of which 381 were females and 453 were males. The pituitaries of only sexually adult specimens were studied for the present report. The specimens were killed by decapitation and the brain along with the pituitary was immediately dissected out and fixed for 24 h in various fixatives, such as, formal sublimate, neutral formalin, Rossman's, Bouin's, Zenker's and Carnoy's fixative. A few glands were immersed in 0·5% and some in 2·5% trichloro acetic acid for 24 h before immersing in formal sublimate. After fixation the pituitary was cut out along with a part of the brain from selected specimens, and the tissues were washed in running water for 24 h, dehydrated by passing through graded ethanol, cleared in xylol and embedded in paraffin. Serial sections of pituitaries were cut at 3–5 μm thickness, some transversely, some horizontally and some sagittally with a view to ascertaining the distribution of the different cell types in the gland. For routine histological study the sections were stained with Ehrlich's or Harris' haematoxylin and counterstained with eosin. Several well established staining procedures were employed to identify the different cell types since specificity was not attainable by any one technique. The different cell types in the adenohypophysis were identified by cross matching by different serial staining procedures. The various gonadotrophs were counted with the help of a Leitz eyepiece grid and a hand tally counter. The cell counts were made from every fifth section and the average per section was arrived at. The total number of cells of a given type in the adenohypophysis was calculated by multiplying the average number per section by the total number of sections.

3. Results

3.1 *Histology and cytochemistry of the pituitary*

3.1a *Neurohypophysis*

(i) *Hypophysial stalk*

The recessus hypophyseus is lined by one or two layers of small rounded darkly staining cells, which are continuous with the ependymal layer of the median eminence. The wall of the hypophysial stalk is composed mostly of numerous parallelly lying nerve fibres which reach the neural lobe. Numerous cells containing nuclei of various shapes and sizes lie scattered irregularly in the stalk. Several blood capillaries lie parallel to the long axis of the stalk between the nerve fibres.

(ii) *Neural lobe*

The neural lobe is composed mostly of nerve fibres which are continuous with the fibres in the hypophysial stalk, but the fibres are less compactly arranged than in the stalk. Whereas the fibres in the dorsal half of the neural lobe lie nearly parallel to the long axis of the neural lobe, many fibres in the ventral half of the neural lobe lie in an

irregular manner. Amidst the nerve fibres occur numerous small glial cells with lightly staining and irregularly shaped nuclei. Some of the cells are large and contain spherical vesicular nuclei. In many places the nerve fibres end in bulbous tips which are intensely PAS-positive. In most cases the end bulbs are surrounded by a few small darkly staining cells. Whereas in the female these are relatively more during late pregnancy and early lactation than during the other phases of the reproductive cycle, and least in number during the non-breeding season, in the male they do not exhibit significant changes in staining characters or numbers during the different phases of the sex-cycle. A few Herring's bodies (figure 4) occur randomly scattered in the pars nervosa. These take a deep bluish-pink stain in sequential AB-PAS-OG procedure. There does not appear to be any change in size and distribution of these during the different phases of the reproductive cycle.

3.1b *Adenohypophysis*

(i) *Pars tuberalis*

This is a poorly vascularised band of cells, 3–4 layers thick, enveloping the pituitary stalk, and this is continuous with the cellular layer covering the base of the median eminence for a short distance. Cytologically, the pars tuberalis contains mostly rounded or oval chromophobes with lightly staining nuclei amidst which lie randomly scattered a few acidophils and basophils whose cytoplasm is OG-positive and OG-PAS-positive respectively. No significant seasonal change was noticed in the cytology of the pars tuberalis correlated with the sexual cycle of the animal.

(ii) *Pars intermedia*

Whereas the pars intermedia is separated from the pars nervosa by a thin, homogeneous PAS-positive and Azan-positive membrane it imperceptibly merges with the pars distalis at the junction of the two on the anterior, posterior and dorso-lateral sides. Small chromophobes form the major cell type of pars intermedia. A PAS-positive Golgi zone containing a glycolipid substance is distinctly demarcated near one pole of the nucleus in these cells. A few colloid cysts occur in some places amidst these cells and larger masses of PAS-positive colloid material occur in the hypophysial cleft (figure 5). Apart from chromophobes two other cell types, each with characteristic tinctorial properties, are present randomly scattered in the pars intermedia. In one type (herein referred to as cell type A) the cytoplasm takes only the PAS stain when stained by PAS-OG sequential staining, has affinity to lead haematoxylin when stained with MacConnail (1947) procedure, to AF in AF-LG-OG and to AB in AB-PAS-OG sequential staining. These cells do not have affinity to OG in any of the sequential staining procedures. Most of these cells are spherical or pear-shaped. Cell type B are intensely AF, PAS, OG and lead haematoxylin-positive. They are normally elongated and thickly fusiform in shape and lie randomly scattered in the pars intermedia. They are markedly smaller and fewer than type A cells. Evidently, the most significant difference between the two types of chromophiles in the pars intermedia is that, whereas type A cells are OG-negative,

type B cells are OG-positive. The exact functional significance of these cells is not known. They do not exhibit any seasonal change in number or staining properties.

(iii)   *Pars distalis*

The chromophobes, which formed the major cellular component, are spherical or pear-shaped cells, each with a vesicular nucleus containing flake-like chromatin material and one or two large spherical bodies, which are deeply stained with haematoxylin and which usually occur near the nuclear membrane. Apart from the chromophobes 5 types of cells exhibiting specific morphological characters and tinctorial affinities with different cytochemical staining procedures could be identified in the pars distalis. Table 1 gives the staining methods employed and the resulting tinctorial properties in the adenohypophysis. Cell types I, II and III are basophils and mucoid, and cell types IV and V are acidophils and non-mucoid. Cell type VI, designated by Van Oordt (1965) could not be identified with any of the cytochemical tests employed in this study including lead haematoxylin (MacConnail 1947) staining procedure.

Cell type I (thyrotrophs—TSH cells) are normally polygonal with the angles being often rounded off (figure 6). The cytoplasm of these cells is finely granular and nucleus vesicular and contains a centrally located nucleolus and 2–4 irregular masses of chromatin material near the nuclear membrane. Visual examination reveals that they increase in number during the beginning of the breeding season in both sexes. Their number appears to become reduced soon after the commencement of gestation in the female and after the regression of the testis in the male.

Cell type II (folliculotrophs—FSH cells) are large gonadotrophs and are usually oval or round in shape (figures 7 and 8). The cytoplasm is finely granular with the granules dispersed uniformly throughout the cell. Vacuoles are commonly noticed in these cells. The cytoplasmic granules are stained blue or purple with sequential PAS-MB-OG procedure. The nuclei take a dark stain with haematoxylin and each contains a well defined dark nucleolus.

Cell type III (luteotrophs—LH cells) could be identified by sequential staining by PAS-MB-OG with which they are stained yellowish-red. These cells are usually round or oval in shape, each containing a large vesicular nucleus with a distinct nucleolus and flake-like chromatin material occurring in two or three groups. The cytoplasm is finely granular or homogeneous (figures 7 and 8).

Cell type IV (lactotrophs—LTH cells) are usually fusiform in shape and each contains a small darkly staining nucleus with clumpy chromatin material usually abutting against the nuclear membrane (figure 9).

Cell type V (somatotrophs—STH cells) are evenly distributed throughout the pars distalis except in the postero-lateral regions, where they occur in slightly larger numbers and in clusters. The cells (figure 10) are round or ovoid and have finely granular cytoplasm. The nucleus is spherical and contains coarse chromatin material occurring in clumps. Visual examination reveals that there is probably a slight increase in their number during late gestation and early lactation. There is no significant difference in their number, location or cytological characters during the different phases of the sex-cycle in the male.

Although the gonadotrophs (cell types II and III) and lactotrophs (cell type IV) are present randomly scattered throughout the adenohypophysis, there is a higher

Table 1. Staining methods employed and the resulting tinctorial properties of the different types of cells in the adenohypophysis.

Staining techniques	Cell type I (TSH cell)	Cell type II (FSH cell)	Cell type III (LH cell)	Cell type IV (LTH cell)	Cell type V (STH cell)	Reference
Modified Mallory triple	Faint green	Faint green	Faint green	Red	Orange	Crossman (1937)
Martius Scarlet blue	Faint green	Faint green	Faint green	Red	Yellow	Lendrum et al (1962)
Carmoisin L-LG-OG	Faint green	Faint green	Faint green	Red	Orange	El Etreby and Tushaus (1973)
LB-PAS-OG	Pink	Pink	Yellow-red	Orange-red	Blue-green	Shanklin et al (1959) and Kerr (1965)
PAS-OG	Red	Pink	Yellowish-red	Orange-red	Orange	Pearse (1968)
AB-PAS-OG	Blue	Bluish-purple	Yellowish-red	Orange-red	Orange	Herlant (1960)
PAS-MB-OG	Pink	Purple	Yellowish-red	Orange-red	Orange	Wilson and Ezrin (1954)
AF-LG-OG	Violet	Faint violet	Faint greenish-blue	Orange-red	Orange	Halmi (1952)
AT-PAS-OG	Blue	Bluish-purple	Yellowish-red	Orange-red	Orange	Ezrin and Murray (1963)

Abbreviations are according to Pearse (1968), Patil (1974) and Richardson (1979).

population of a given type of cell aggregated in patches in certain regions of the adenohypophysis. Further, the regional aggregation of the different gonadotrophs and lactotrophs appears to be specific and constant throughout the year although the total number of a given type of cells varies not only among the randomly distributed cells but within each region of aggregation during different phases of the sex-cycle. For example, the LTH cells occur in a randomly scattered manner throughout the pars distalis, but they also occur in large numbers and in closely aggregated groups along the borders of the hypophysial cleft and a few regions in the mass of the pars distalis. Their absolute number varies during different seasons, but the relative population in the different regions remains nearly the same during all the phases of the reproductive cycle. This applies to the gonadotrophs also, the location of the aggregated groups of these cells, however, differs. Figures 1 and 2 give the
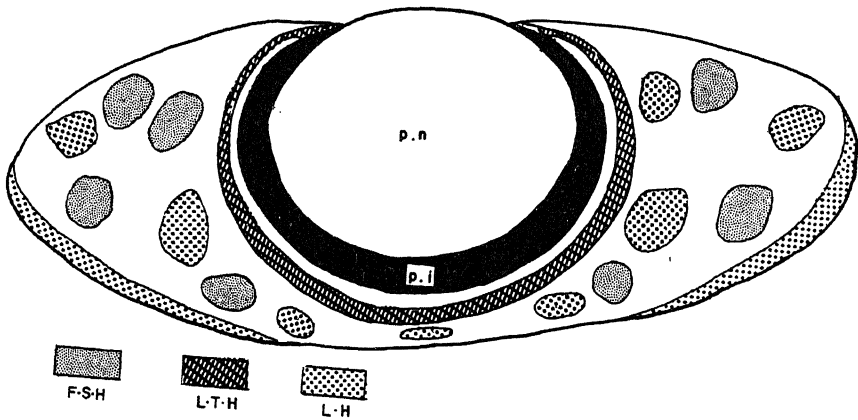
Figure 1. Schematic representation of transverse section of the pituitary to show the distribution of patches. (p.i, Pars intermedia; p.n, pars nervosa).
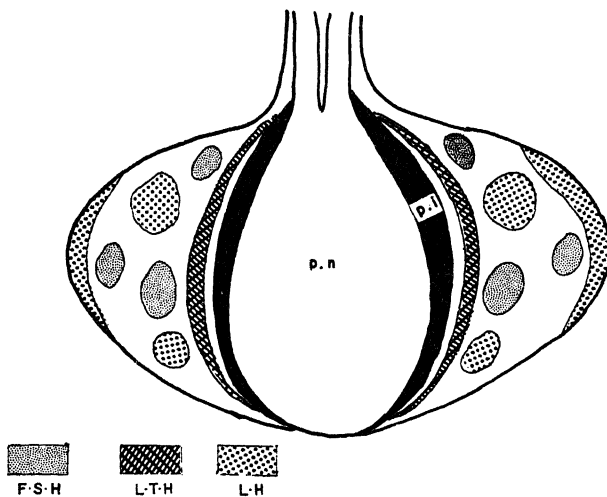
Figure 2. Schematic representation of the horizontal longitudinal section of the pituitary to show the distribution of patches of gonadotrophs and lactotrophs.

distribution of the patches of aggregations of different types of gonadotrophs and lactotrophs in the pars distalis in the female.

3.2 *Cyclical changes in the gonadotrophs and lactotrophs*

There are marked differences in the total number of different kinds of gonadotrophs and lactotrophs during different phases of sexual cycle of this bat. Table 2 gives the number of different kinds of gonadotrophs and lactotrophs during different phases of the sexual cycle of the female and figure 3 is a graphic representation of the changes in the numbers of the gonadotrophs and lactotrophs during the different phases of the female sexual cycle of this bat. The table and graph indicate that FSH cells commence to increase in number from prooestrus and reach the maximum number at oestrus after which they decrease in number until the next cycle. LH cells commence increasing in number a little after prooestrus and reach maximum values

Table 2. Total cell counts of different gonadotrophs and lactotrophs during different phases of reproductive cycle of female.

Phase of cycle	FSH cells	LH cells	LTH cells
Anoestrus	27720	28497	52350
Prooestrus	33560	36330	53016
Oestrus	174660	90240	75812
Early pregnancy	106080	142912	98250
Mid-pregnancy	41448	35620	100180
Late pregnancy	34650	53400	145300
Early lactation	29260	36800	206128
Late lactation	20254	31395	178526
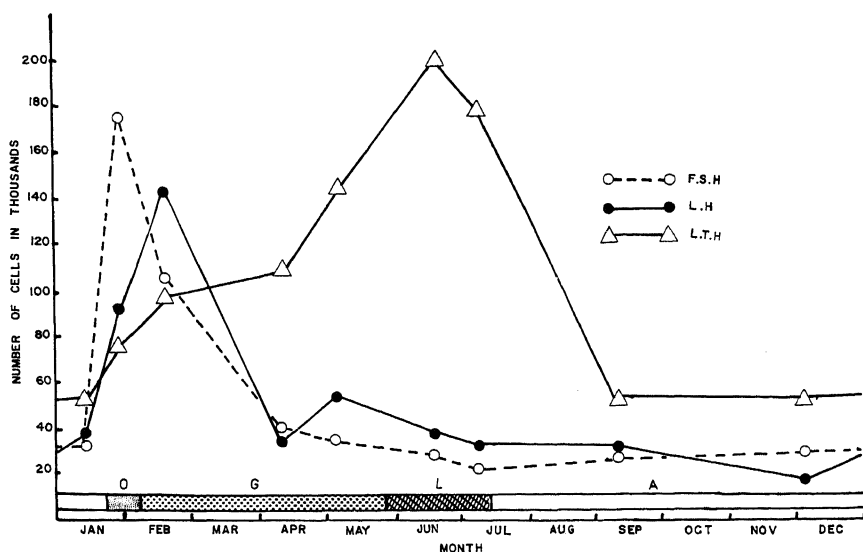
Figure 3. Graph to show the numerical changes of gonadotrophs and lactotrophs during different months of the year.
(A, Anoestrus; G, gestation; L, lactation; O, ovulation and fertilization).
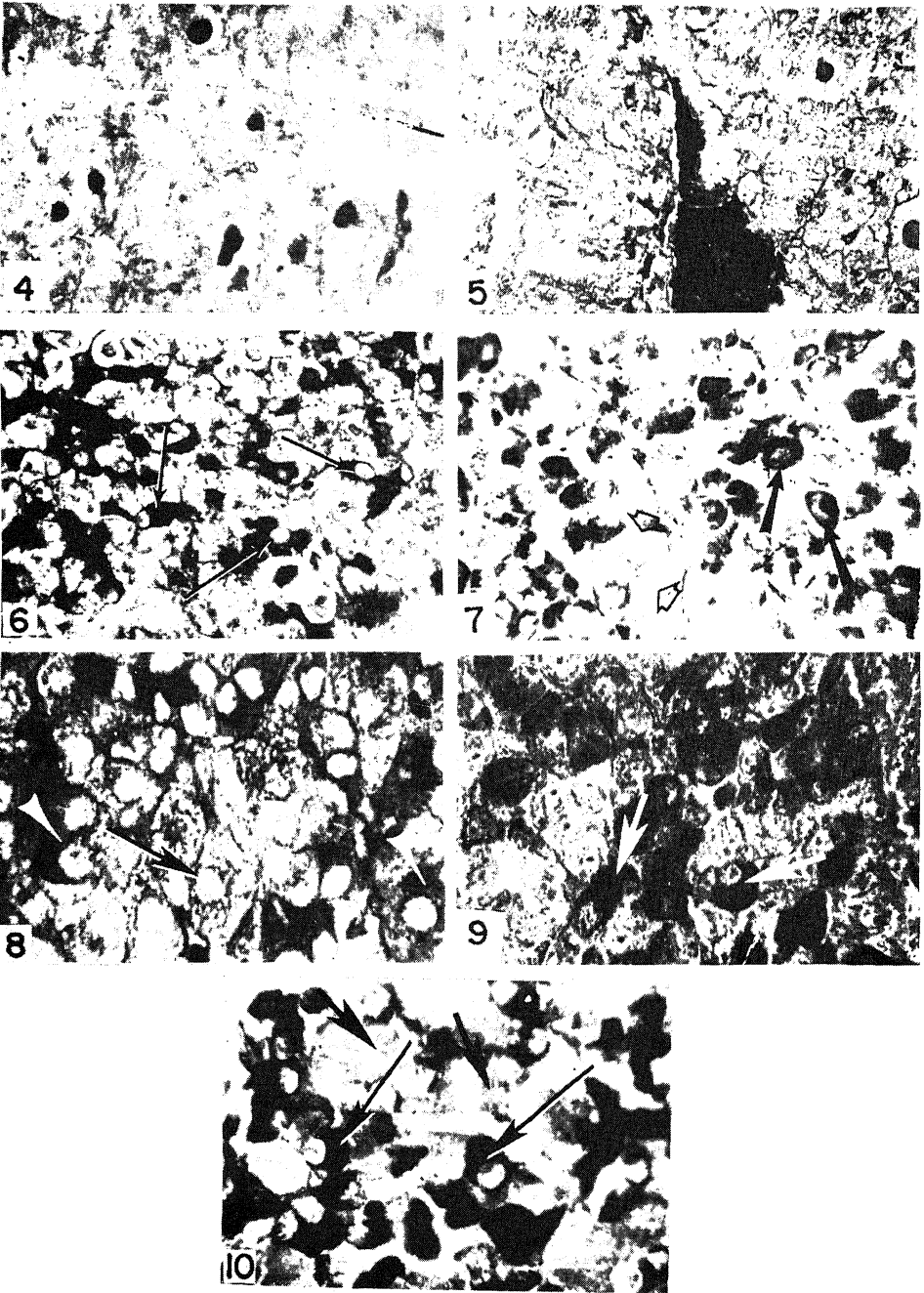
Figures 4–10.

ring early pregnancy after which they decrease in number rapidly until about mid-
egnancy when there is slight increase in their number. During advanced pregnancy
ey become reduced in number until the following sexual season. The LTH cells
ogressively increase in number from early gestation until early stages of lactation
en they reach their maximum numbers. Thereafter they become reduced progres-
ely in number and reach low levels after the cessation of lactation.

The males also exhibit marked variations in the number of different types of
nadotrophs and lactotrophs during the sexual cycle. Table 3 gives the total counts
the different types of gonadotrophs and lactotrophs during the sexually active and
active periods of life. The counts were made once during the time when the testis
hibited the highest peak of spermatogenetic activity, and a second time when the
stis was inactive. From the table it is evident that there is an increase in the number
both the types of gonadotrophs and the lactotrophs during the period when the
stis and accessory glands of the males are at peak stage of activity.

Discussion

e tinctorial affinities of the different cell types and their interpretation in relation
the endocrine physiology of these cells have been discussed in considerable detail
Patil (1974) and Richardson (1978, 1979). Whereas Van Oordt (1965) classified
ese and implied that each cell type produces one hormone, the one-cell-one-
rmone hypothesis was contested by some workers (Nakane 1970; Herbert 1976;
loriarty 1976; Richardson 1979) who, on the basis of immunohistochemical studies
these cells in the pars distalis, indicated that more than one hormone was
roduced by the same cell. Notwithstanding this difference of opinion, the fact that
ere are definite cyclical changes in the number and structure of the different types
cells in consonance with the sexual cycle of most animals studied so far, is a strong
rcumstantial evidence suggesting that the different types of cells identified on the

Table 3. Total cell counts of the gonadotrophs and lacto-
trophs during sexually active and inactive period of male.

State of sexual activity	FSH cells	LH cells	LTH cells
Inactive	17810	14352	18228
Active	45927	87856	200032

Figures 4–10. 4. Part of the neural lobe to show a few Herring's bodies (AB-PAS-OG
staining) (blue filter) (× 180). **5.** Part of the section passing through pars intermedia and
pars distalis. Note the large colloid mass in the hypophysial cleft (PAS-MB-OG staining)
(blue filter) (× 135). **6.** A part of the pars distalis of female in anoestrus. Arrow points to
TSH cells (AF-LG-OG staining) (blue filter) (× 285). **7.** Pars distalis of female during mid-
pregnancy. Note LH cells (hollow arrows) and FSH cells (thick arrows) (AB-PAS-OG
staining) (yellow-blue filter) (× 360). **8.** Pars distalis of male during active breeding season.
Note two types of gonadotrophs—LH cells (white arrowheads) and FSH cells (shaded
arrow) (PAS-OG staining) (yellow filter) (× 360). **9.** Pars distalis of female during early
pregnancy. Note prominent LTH cells (white arrows) (CL-LG-OG staining) (yellow filter)
(× 360). **10.** Pars distalis of female during lactation. Note the STH cells (long shaded
arrow) and LTH cells (short black arrow) (LB-PAS-OG staining) (yellow filter) (× 300).

basis of their tinctorial affinities are involved in the endocrine functions assigned to them. This conclusion is not vitiated even if more than one hormone is produced by some of the cells. On the other hand it gives additional support to the contention that the cells do undergo changes depending on the physiological need of the animal. The present study demonstrates that the different types of gonadotrophs and lactotrophs in the adenohypophysis in both sexes undergo changes in number and cytological characters parallel with the reproductive periodicity of *T. melanopogon* (Sapkal and Khamare 1984).

One question, however, remains unanswered so far. This relates to the method by which an increase in the number of different cell types during different phases of the reproductive cycle is brought about. Mitotic divisions of the cells of the pars distalis of adult mammals have not been so far demonstrated conclusively. In the absence of cell divisions, there can be two possible explanations for the periodic increase and decrease in the number of different cell types. Either the cells undergo inter-conversion depending on the functional need of the animal, or the chromophobes become differentiated into different cell types depending on the physiological demand and revert into chromophobes after the period of demand.

Acknowledgements

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Haemodynamics of hamsters during *Ancylostoma ceylanicum* infection

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Abstract. Alterations in haematological and liver glycogen values during the course of *Ancylostoma ceylanicum* ($100L_3$, p.o.) infection in hamsters were investigated. In early phase of infection, there were marginal changes in haematological parameters, albumin-globulin ratio and liver glycogen, which transformed into severe alterations on patency (day 18, post infection). Haemoglobin, packed cell volume, total erythrocyte counts and liver glycogen values had a decreasing trend and reticulocytes increased considerably to compensate the red blood cell loss. Albumin/globulin ratio which was in favour of albumin in the beginning, reversed on day 24 p.i. When the animals were deparasitized with mebendazole (5 mg/kgxl, p.o.), all the parameters started resuming normalcy and on day 12 of treatment of all the parameters except albumin/globulin ratio attained the pre-infection level.

Keywords. *Ancylostoma ceylanicum*; hamster; haemodynamics; liver glycogen.

1. Introduction

Infection with gastrointestinal helminths inflict a variety of structural and functional changes in the intestine (Symons 1969) and blood of hosts (Ogilvie *et al* 1978; Roth and Levy 1980). Qualitative and quantitative changes in haematological picture constitute some of the most frequent and easily observed manifestations of infection and also reflect the immune response of the host to infection (Boyer *et al* 1971; Ogilvie *et al* 1978; Moqbel 1980). Since, *Ancylostoma ceylanicum* a hookworm of dog, cat and man is of relatively recent introduction to the experimental field (Ray *et al* 1972; Visen *et al* 1984; Gupta *et al* 1987; Srivastava *et al* 1986) and gained favour for experimentation over other hookworm models, *Nippostrongylus brasiliensis* in rats and *Nematospiroides dubius* in mice (Ray *et al* 1972; Misra *et al* 1981; Katiyar *et al* 1982, 1984) there was need to understand haematological and related parameters of the infected host (hamster). A detailed study on this aspect is also important because this laboratory host is the most suitable for *A. ceylanicum*.

Accordingly an attempt has been made to monitor alterations in haematological parameters and liver glycogen reserve of hamsters during the course of infection and following anthelmintic therapy.

2. Materials and methods

2.1 *Animals*

Laboratory bred male hamsters (70–85 g) maintained under standard animal house conditions, served as experimental host.

2.2 *Ancylostoma ceylanicum*

The procurement of infective larvae, infection to host and recovery of worms have already been dealt in detail elsewhere (Gupta *et al* 1987; Srivastava *et al* 1986).

2.3 *Experimental design*

Two groups of animals were examined. Group I (infected) comprising of 60 hamsters, was infected orally with $100 \pm 5L_3$. Three of the survivers from this group were exsanguinated at 3 days interval from day 3–30 p.i. (10 intervals) and their blood samples were collected for examining haematological parameters [Haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), packed cell volume (PCV), reticulocyte percentage (RP), differential leucocyte count (DLC) and albumin/globulin ratio (A/G)]. Liver was isolated under chilled condition for estimating glycogen.

On day 18, when the infection attained patency (Visen *et al* 1984; Gupta *et al* 1987) in the intestine, the remaining animals of Group I (infected) were divided into two sub groups. The animals of one sub group were deparasitized with mebendazole (5 mg/kgxl, per os) and those of the other sub group received sham treatment of normal saline and examination continued till 30 p.i.

Group II (uninfected) having identical number of animals and receiving mebendazole and sham treatment of D.W. was run parallel and examined simultaneously.

On autopsy after collecting blood and liver; intestine of each animal was also examined for observing the worm load. Two to three replicates were carried out.

2.4 *Haematological examination*

Hb was determined by Sahli-Hellige Haemoglobinometer. Total erythrocyte and leucocyte counts were made on heparinized blood using standard dilution methods (Hayem's fluid for RBC and Turk's fluid for WBC) on a Neubauer haemocytometer (Anon 1978). The differential leucocyte count was made on films of non heparinized blood, fixed in methanol and stained with 10% Giemsa stain. Packed cell volume was determined with heparinized blood in Wintrobe tubes (Anon 1978). The reticulocytes were counted as described by Cheema and Scofield (1984).

2.5 *Determination of A/G ratio*

The test sera were first electrophoresed on cellulose acetate membrane in microzone electrophoretic apparatus (Beckman) (Briero and Mull 1964; Jane 1979) and later were scanned over the scanning densitometer (R-112) at 520 nm.

2.6 *Estimation of liver glycogen*

Liver glycogen (LG) was extracted from 25–50 mg isolated liver (Good *et al* 1933) and estimated colorimetrically (Montgomery 1957).

Results

The sequential changes in various parameters that occurred during the course of infection have been depicted in table 1.

3.1 *Hb*

Hb level remained unaltered upto day 6 of infection. On day 9 slight decrease was seen which became pronounced as the infection reached patency i.e. on day 18. Maximum drop was observed on day 24 of infection (infected group-4·50 ± 0·94 g%, control group-17·59 ± 0·98 g%).

Mebendazole, knocked out all the worms, and Hb started rising from day 3 post treatment and reached pre-infection level on day 12 post treatment.

3.2 *TEC*

TEC showed a gradual fall which was significant on day 12 (when L_5 stage was present). On day 18 when adult worms started sucking blood, the fall was more conspicuous and maintained a plateau upto day 30 (last observed period).

3.3 *TLC*

Very mild alteration was recorded and the maximum rise (46·5% over the control) was observed on day 12 of infection.

3.4 *DLC*

The differential count remained more or less unaltered during the course of infection.

3.5 *PCV*

Decrease in PCV started from day 9 and continued till the day of last observation (day 30 p.i.) where the value of infected group was 8·25 ± 1·09% as compared to 48·39 ± 5·31% in control group.

3.6 *RP*

The reticulocytes were very few in uninfected hamsters (mean value-1 ± 0·68%) but in the infected animals, a dramatic rise was recorded from day 15 of infection and attained peak (i.e. 78 ± 3·74%) on day 30 p.i.

3.7 *A/G ratio*

The A/G ratio which in the initial phase was in favour of albumin (mean value 1·47 ± 0·52) levelled off on day 15 p.i. (mean value 1·05 ± 0·19) and subsequently turned in favour of globulin on day 24 (0·88 ± 0·14).

Table 1. Dynamics of blood parameters and liver glycogen reserve of male hamsters during *A. ceylanicum* (100 L$_3$/p.o) infection.

Parameters observed (pooled control values) Group II	Group I	Observation intervals (days)									
		3	6	9	12	15	18	21	24	27	30
Hb g% (17·59±0·98)	Infected	16·73±0·81	16·70±1·31	14·63±1·17	10·57±0·77	8·30±1·13	5·11±1·94	4·52±0·90	4·50±0·94	4·60±0·5	4·60±0·37
	Infected treated	—	—	—	—	—	—	8·88±2·05	10·3±2·69	15·1±2·80	18·1±1·1
TEC (×10⁶) (6·13±0·42)	Infected	5·40±0·09	5·39±0·10	5·64±0·19	3·42±0·25	2·65±0·19	1·45±0·45	1·26±0·47	1·09±0·16	0·97±0·14	1·07±0·19
	Infected treated	—	—	—	—	—	—	3·45±0·53	3·39±0·26	4·8±1·24	5·09±0·47
TLC (5189±1475)	Infected	5637±543	5859±421	5791±264	7607±393	5908±175	6427±1006	5557±1581	6250±425	5050±1148	5437±499
	Infected treated	—	—	—	—	—	—	5808±896	5587±589	5800±699	5000±331
PCV % (49·39±5·31)	Infected	48·83±0·95	49·0±2·15	39·66±4·41	25·67±1·74	19·50±0·66	13·75±3·9	12·43±3·58	9·33±1·49	9·17±1·95	8·25±1·09
	Infected treated	—	—	—	—	—	—	27·33±3·25	35·5±2·29	40·5±6·73	45·0±5·6
Reticulocyte % (1·00±0·68)	Infected	0·90±0·2	0·67±0·32	1·66±0·45	4·58±2·04	33·15±2·71	67·02±10·01	61·45±11·32	57·92±3·37	59·79±0·9	78·0±3·74
	Infected treated	—	—	—	—	—	—	28·66±16·83	5·65±1·75	2·63±1·04	1·49±0·39
A/G (1·50±0·35)	Infected	1·47±0·52	1·38±0·65	1·35±0·21	1·17±0·32	1·05±0·19	1·01±0·15	1·02±0·31	0·88±0·14	0·80±0·5	0·92±0·06
	Infected treated	—	—	—	—	—	—	1·07±0·11	1·10±0·19	0·97±0·05	0·97±0·23
Liver glycogen mg/g (74·73±8·76)	Infected	68·86±4·37	66·27±13·50	39·39±7·61	26·74±3·50	17·63±5·88	8·17±5·76	1·25±0·72	2·25±0·75	1·84±1·38	1·90±0·75
	Infected treated	—	—	—	—	—	—	16·77±4·9	51·87±4·5	58·41±18·04	75·53±5·42

Mean value ± SE.

3.8 *LG*

From day 6 of infection, there was gradual depletion of glycogen reserve from the liver and on day 9, almost half of the reserve was exhausted (39.39 ± 7.61 mg/g vs 74.73 ± 8.76 mg/g). The depletion was proportionate to the age of infection and almost all the glycogen was utilized by day 27 p.i. (1.84 ± 1.38 mg/g).

4. Discussion

Haematological changes represent a facet of the host's overall response to infection. In gastrointestinal helminthiases this aspect has been well documented, firstly from a description point of view (Ruitenberg *et al* 1977; Moqbel 1980; Ogilvie *et al* 1980; Przyjalkowski 1980; Cheema and Scofield 1984) and secondly in an attempt to pinpoint the cells that may be important for host defence (Ogilvie *et al* 1980; Wakelin and Donachie 1983).

The changes that occurred in the TEC, PCV and Hb parameters suggested the development of microcytic anaemia which is characteristic of blood sucking hookworms (Chatterjee 1967; Roach 1970; Hoagland and Schad 1978). These parameters showed the abducting trend after day 12 of infection when cutting armature of parasite was fully developed and they started removing the blood.

The marked increase in reticulocytes during patency may be due to heavy destruction of erythrocytes, which consequently forced the reticulocytes to accumulate in the blood to compensate erythrocytic loss.

The elevated leucocytes in early phase of infection was in conformation with the previous observations of Baker (1962), Ogilvie *et al* (1978) and Roth and Levy (1980) but dipping at the later stage of infection could be due to the severe loss of cellular components of the blood because of haemophagy by parasite.

The reversion of A/G ratio at the later stage of infection depicted the development of protective immunity (Gupta and Katiyar 1985). Carroll and Grove (1985) also observed increased immunoglobulins in dogs following *A. ceylanicum* infection. Non resuming of A/G ratio after mebendazole therapy may be explained on the basis of definite half life of antibodies. Therefore, as soon as the antigenic source is removed, the globulin levels are not supposed to reach preinfection level.

Excess of carbohydrate is stored in the liver as glycogen and is released during emergencies. The excessive blood loss during *A. ceylanicum* infection and consequent drainage of glucose along with, necessitated an additional demand of glycose by the host. Hence, the liver glycogen was mobilized to meet the host requirement (Kaul *et al* 1982). As such, the reserve glycogen in the liver was depleted and showed decreasing pattern.

It may be inferred that *A. ceylanicum* like other hookworms of men and animals, is highly pathogenic to its laboratory host, hamster, suggesting the suitability of this host parasite combination for experimental studies.

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Specialised integumentary glands of the Indian field mouse, *Mus booduga booduga*

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Abstract. Histomorphological investigations on the specialised integumentary glands of Indian field mouse, *Mus booduga booduga* have revealed the existence of hypertrophied integumentary glands in the eyelids (tarsal), oral angle and at the perineal (circumanal) regions. The histophysiological characteristics of these specialised integumentary glands and their probable behavioural significance are discussed.

Keywords. Indian field mouse; *Mus booduga booduga*; specialised integumentary glands; eyelid (tarsal); oral angle; perineal (circumanal); holocrine sebaceous; behavioural relevance.

1. Introduction

Chemical signals play a prominent role in the modulation of behavioural responses of mammals. The specialised skin glands constitute the prime sites of production of olfactory cues and have an ubiquitous occurrence in mammals (Quay 1959; Muller-Schwarze 1967; Balakrishnan and Alexander 1976, 1977a, b; Bhaskaran and Alexander 1985). Based on their mode of secretion, these glands have been categorised into 3, holocrine sebaceous glands, apocrine and eccrine sweat glands. These glands have been extensively investigated in murine and microtine rodents and different types of glands have been reported (Quay 1953, 1954a, b; Quay and Tomich 1963; Stoddart 1972). However Stoddart (1974) had reported that mice of the genus *Mus* do not possess discrete scent organs of behavioural relevance.

Our investigations on the ethology of the Indian field mouse, *Mus booduga booduga*, revealed that it is a nocturnal burrowing species with a relatively complex social structuring of the natural population exhibiting a social order warranting a complex communicating system. It has been observed that during the social interactions of the Indian field mouse, olfactory cues play a very important role with the major sites of body odour being frequently investigated by the interacting conspecifics concerned. Accordingly a study has been undertaken to elaborate the histophysiology of the specialised skin glands of these animals.

2. Materials and methods

Freshly trapped, healthy adult Indian field mice, *M. b. booduga* of both sexes were sacrificed by overetherisation. After shaving off the hair, skin samples were carefully excised from various regions of behavioural relevance. Skin from adjoining regions

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were also removed for comparison with typical sebaceous and sudoriferous glands. The tissue was fixed in Bouin's fluid, dehydrated in alcohol series, embedded in paraffin and sections of 6 μm were cut. The paraffin sections were stained in Ehrlich's hematoxylin and eosin.

3. Results

The histological investigations revealed that highly developed integumentary glands exist at the oral angle, eyelid and perineal regions.

Sebaceous glands generally appear always associated with the hair follicles. Sebaceous acini have compact cells with intensely staining cytoplasm with a centrally located nucleus, which are the young holocrine cells. Further, diffusely staining mature cells could also be discerned with a frothy cytoplasm due to numerous minute oil droplets. The sudoriferous glands are comprised of large well developed coiled glandular tubules formed of a single layer of cubical epithelial cells. The larger central lumen is rather clear with secretory material found either attached to the walls of the tubules on the inner aspects as a thin layer of cytoplasm or found scattered in the lumen. The sudoriferous glands are apocrine in nature.

3.1 *The eyelid gland (tarsal gland)*

There is an accumulation of large sebaceous follicles at the inner corner of the eyelids and these extend into the eyelids (figure 1). The gland has a distinct duct which voids to the exterior at the inner corner of the eye (figure 2). The sebaceous acini are not associated with the eyelashes or the hair follicles on the adjacent skin. Each lobe of the glandular region extends into each eyelid filling up the space between the outer and inner epidermal layers of the eyelid. The gland extends along the entire length of the eyelid with the maximum number of lobes ranging up to 49 in males and 19 in females, being located at the inner corner of the eyelid. These lobes have a distinct large common duct into which the smaller ducts from the lobes drain their contents (figure 2).

3.2 *Oral angle gland*

The oral angle region has highly modified sebaceous glands as well as well developed sudoriferous glands in *M. b. booduga*. The oral angle sebaceous glands generally do not open into the hair follicle, quite contrary to the natural pattern of such glands. There are two closely situated but distinct sebaceous glandular masses, one located on the cheek wall and the other at the oral angle. The former is located at a larger area, relatively less compact and diffuse, comprised of well developed sebaceous acini embedded in the cheek musculature (figure 3). It is continous with the vibrissal hair follicles. There is no common duct but different follicular aggregations void the exudates separately to the outer skin surface. The latter is clearly delineated compact area with a few associated hair follicles. It voids into the oral angle through a distinct duct originating from a central lacuna. The glandular zone does not exhibit any externally visible morphological boundary. The cells of these two glandular zones are characteristically sebaceous glands having large
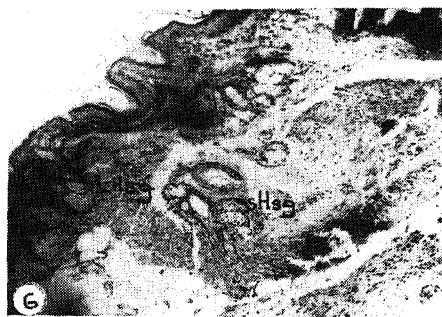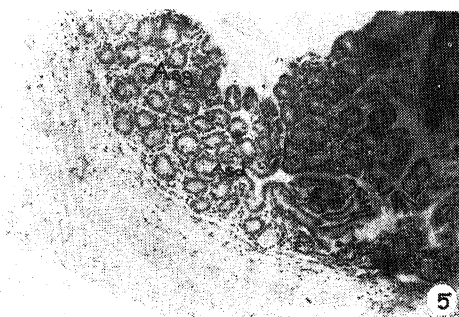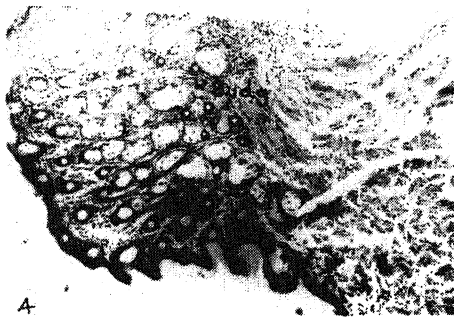
Figures 1–6. **1.** Vertical section through the eyelid of a male field mouse showing the hypertrophied sebaceous glands (× 100). **2.** Vertical section through the eyelid of a female field mouse showing the smaller ducts coalescing to form the large duct which opens to the exterior at the inner corner of the eye (× 100). **3.** Hypertrophied sebaceous glands at the oral angle (× 100). **4.** Sudoriferous glands found at the oral angle (× 100). **5.** Transverse section through the rectum (× 400). **6.** Perineal region showing the large and small groups of sebaceous glands (× 400).

(lsg, Large sebaceous follicles; sgd, sebaceous glandular duct; lcd, large common duct; nsg, normal sebaceous glands; Hsg, hypertrophied sebaceous glands; Sudg, sudoriferous gland; Asg, apocrine sudoriferous glands; Asd, apocrine sudoriferous glandular duct; LHsg, large hypertrophied sebaceous glands; sHsg, small hypertrophied sebaceous glands).

spherical cells, with a large central nucleus and sebum filled cytoplasm. Younger acini stain more intensely because of their dense cytoplasm whereas mature cells have a pale appearance due to the accumulated lipoid secretions therein.

Well developed sudoriferous glands are also seen interspersed between sebaceous

acini. The glands are coiled and tubular and void. their secretions to the exterior. The epithelial wall of each tubule is formed of a single layer of cuboidal or columnar cells (figure 4).

3.3 *Perineal glands*

Two types of modified sebaceous glands as well as apocrine sudoriferous tubules have been located in the mucocutaneous junction of the perineal region: (i) Large perineal sebaceous glands located at the anal papilla with younger acini being found adjacent to the inner proctodeal lining and the large sebum filled mature acini near the outer edge (figure 5). (ii) The smaller sebaceous glands are located in the deeper layers of the skin (figure 6). The individual acini tend to open into the rectum as well as into smaller ducts which coalesce to form larger ducts which open into the anal cleft.

The apocrine sudoriferous glands could be seen as tubular coiled structures. These glands drain their secretions to the exterior by separate constricted ducts.

4. Discussion

Olfactory cues have a signatory role in influencing the adaptive responses in nocturnal animals. Specialised integumentary glands of vertebrates are conveniently located for the selective dissemination of chemical signals into the environment. Thiessen and Rice (1976) demonstrated that skin glands in rodents such as Mongolian gerbil, Syrian hamster and deer mice are behaviourally significant. Stoddart (1974) reported that there are no specialised integumentary glands of behavioural relevance in mice belonging to the genus *Mus*.

However data obtained during the present study indicate that the highly developed integumentary glands of *M. b. booduga* are specifically localised at the eyelid (tarsal), oral angle and perineal regions. Behavioural observations on this animal suggest that their specialised glandular secretions may probably function as olfactory cues. In fact during their social interactions, the conspecifics exhibit specific behavioural responses, investigating the olfactorily relevant glandular zones quite frequently. The major zones of olfactory relevance, being located at the mouth corner, eye and anal region, elicit behavioural responses such as naso-nasal sniffing, oro-oral contact and naso-perineal investigations.

Autogrooming is a commonly observed behavioural response in *M. b. booduga*, whereby the glandular regions are frequently groomed, so that the glandular secretions are anointed all over the body. The oral angle and tarsal glands in *M. b. booduga* appear to be more involved in social interactions than the perineal glands. As Kivett (1975) and Kivett *et al* (1976) pointed out, the mouth corner glands of the ground squirrels, which are deployed in substrate marking (mouth rubbing) probably convey social cues, such as group membership, identity and social status. Studies on the Indian palm squirrel, *Funambulus palmarum* had shown that the oral angle glandular secretions are concerned with agonistic behaviour (Bhaskaran and Alexander 1985). During social interactions, the perineal glands of *M. b. booduga* are constantly subjected to olfactory investigations. Although no active scent marking such as perineal drag and anal rubbing has been observed in Indian field

ce, there is a definite possibility of these glandular secretions being mixed with
ne and faeces. The frequency of anal sniffing was quite high between sexes and
s suggests that the perineal glandular secretions could be concerned with
luation of reproductive and sexual status.

The present study has explicitly demonstrated the presence of specialised
egumentary glands of behavioural relevance at eyelid (tarsal), oral angle and
rineal regions. Further studies on the probable behavioural relevance of the
ndular secretions of these specialised integumentary glands are in progress.

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Bioenergetics and reproductive efficiency of *Atractomorpha crenulata* F. (Orthoptera : Insecta) in relation to food quality

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Abstract. Food consumption and utilization efficiency of *Atractomorpha crenulata* feeding on the leaves of *Ricinus communis, Arachis hypogaea* and *Panicum maximum* are discussed. Longevity and fecundity of the insect feeding on *Ricinus communis* were higher as compared to feeding on the other plants. Females tend to consume more food than the males in all the tested food plants. Energy allocated by the female to egg production varied between 7 and 15% of the assimilated energy during the adult phase.

Keywords. Food quality; food utilization; reproductive efficiency; *Atractomorpha crenulata*.

1. Introduction

Information on the quantitative food consumption and utilization of insects has been reviewed by Slansky and Scriber (1985) and Muthukrishnan and Pandian (1987a). Most of the relevant publications on the bioenergetics of insects relate to lepidopteran larvae. While basic information on the bioenergetics of acridids is provided by Delvi and Pandian (1971, 1972), the present work attempts to correlate the effect of food quality on food consumption and utilization of *Atractomorpha crenulata*, a phytophagous acridid weighing 0·4 g. As the reproductive efficiency of insects depends upon their life style and feeding pattern (Muthukrishnan and Pandian 1987b), different strategies are adopted by different insects to maximise their investment on reproduction (Calow 1973; Thompson 1975). In insects, the energy allocated to reproduction varies from 2–81% of their consumed energy (Llewllyn and Qureshi 1978; Slansky 1980). While Ananthakrishnan *et al* (1985) have considered the effect of food quality (*Ricinus communis, Arachis hypogaea* and *Panicum maximum*) on food consumption, longevity and fecundity of *A. crenulata* on dry weight basis for 24 h period with special reference to sensory morphology, the present paper reports the reproductive efficiency and food utilization efficiencies of *A. crenulata*.

2. Materials and methods

Eggs of *A. crenulata* reared in the laboratory were obtained from the soil before hatching. The newly emerged nymphs were weighed individually and reared in plastic terraria (375 ml capacity). The nymphs were fed *ad libitum* on weighed quantities of the leaves of the food plant (*R. communis, A. hypogaea* and *P. maximum*), twice a day. To serve as control, a sample leaf of identical age was dried at 60°C to constant weight in order to estimate the water content (Waldbauer 1968). Every day before feeding, unfed remains and the faeces egested by the insect were removed and

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dried for each instar and the adult. The nymphs were weighed after moulting into the successive instar as well as the adult female during the oviposition period which was determined by the development of oocyte in the ovary (Murugan and Jacob 1988), whereas food consumption by the male was estimated once in 30 days. Eggs were collected from the wet soil provided in the terraria in specimen tubes (5×5 cm). Following the gravimetric method of Waldbauer (1968) food consumption (C), egestion (FU) and growth (P) were estimated. Energy contents of the food, faeces and insect were estimated in a Parr 1421 semi-micro bomb calorimeter following the standard procedure described in the instruction manual. The increase in temperature due to the burning of the sample was recorded in a Omniscribe[R] recorder. Once in five estimations, the bomb was standardised by using benzoic acid. Using these energy values the mass budget involving C, FU and P was converted into energy budget expressed in Joules. Growth was estimated as the difference between the energy content at the commencement of an instar or the adult stage and at the end of that particular stage.

Bioenergetics parameters were estimated following the IBP formula of Petrusewicz and Macfadyen (1970)

$$C = FU + P + R. \tag{1}$$

Assimilation was calculated as the difference between food consumed and that of faeces;

$$A = C - FU, \tag{2}$$

metabolism was calculated as the difference between assimilated energy and that of growth

$$M = A - P. \tag{3}$$

Rates of feeding (Cr), assimilation (Ar), production (Pr) and metabolism (Mr) were calculated by dividing the respective quantitative values expressed on per insect basis by the mid body weight of the live insect (g) and duration of the instar/adult stage expressed in J/g live insect/day (Waldbauer 1968). The following formulae were used to compute the rates and efficiencies:

$$Cr = \frac{\text{Consumption (J/insect)}}{\text{Mid body weight (g)} \times \text{duration of the instar/stage}} \tag{4}$$

$$Ar = \frac{\text{Assimilation (J/insect)}}{\text{Mid body weight (g)} \times \text{duration of the instar/stage}} \tag{5}$$

$$Pr = \frac{\text{Production (J/insect)}}{\text{Mid body weight (g)} \times \text{duration of the instar/stage}} \tag{6}$$

$$Mr = \frac{\text{Metabolism (J/insect)}}{\text{Mid body weight (g)} \times \text{duration of the instar/stage}}. \tag{7}$$

Assimilation efficiency (Ase) was calculated in percentage relating energy assimilated (A) to that of ingested (C). Production efficiencies (Pe_1 and Pe_2) were calculated in percentage relating production (P) to ingestion (C) and assimilation (A).

Overall rates were calculated by dividing the sum of products of the respective rates and the duration for the different life stage by the total number of days of the feeding period as given below:

$$\text{Overall } Cr = \frac{\text{Sum of production of } Cr \text{ and duration for each instar/stage}}{\text{Total feeding period (day)}} \tag{8}$$

$$\text{Overall } Ar = \frac{\text{Sum of production of } Ar \text{ and duration for each instar/stage}}{\text{Total feeding period (day)}} \tag{9}$$

$$\text{Overall } Pr = \frac{\text{Sum of production of } Pr \text{ and duration for each instar/stage}}{\text{Total feeding period (day)}} \tag{10}$$

$$\text{Overall } Mr = \frac{\text{Sum of production of } Mr \text{ and duration for each instar/stage}}{\text{Total feeding period (day)}} \tag{11}$$

Reproductive efficiency (%) was calculated by dividing the energy allocated to egg production to that of energy assimilated during their adult phase (Muthukrishnan and Pandian 1987a)

$$RE (\%) \frac{\text{Energy allocated to egg production (J/insect)}}{\text{Total energy assimilated during the adult phase}}. \tag{12}$$

Results

1 Chemical composition and growth

Nitrogen and energy contents of *R. communis*, *A. hypogaea* and *P. maximum* were ·52% and 23·282 J, 1·92% and 19·834 J, and 1·64 and 17·538 J; while water content was 75·2, 68·9 and 66·4%, respectively. Newly moulted I instar nymph of *A. crenulata* weighed 1·7 mg and contained 25·6 J. With progress in development, energy content of the nymph increases exponentially. For instance, energy content of newly emerged adult female and male feeding on *R. communis*, *A. hypogaea* and *P. maximum* was 922·5 and 751·3 J, 851·9 and 714·5 J and 608·3 and 423·7 J, respectively. Developmental period of a female nymph varied from 39·8 days while feeding on *R. communis* to 44·3 days on *P. maximum*. The number of ovipositions decreased from 6 in castor leaf feeding schedule to 4 or 3 in less preferred host plants. Length of life span of *A. crenulata* fed on *R. communis* was 169 days but decreased to 112 days in *P. maximum* feeding regime. Growth of the nymph increased exponentially with advancing age. For instance, I instar nymph feeding on *R. communis* leaf contained 26·5 J grows to 922·5 J during adult stage (figure 1).

2 Bioenergetics

Food energy ingested by the insect increased with advancing life stage upto oviposition period and then decreased. For example, food consumption of *A. crenulata* feeding on the leaf of *R. communis* increased from 851 J (I instar) to 471 J (I oviposition period), and decreased to 6385 J during the last oviposition
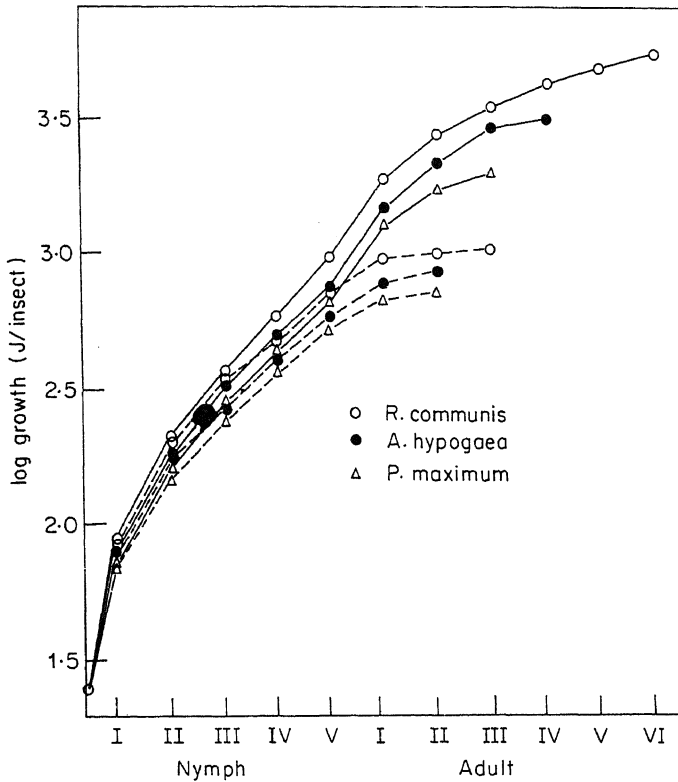
Figure 1. Growth of *A. crenulata* male (broken line) and female (continuous line) fed *ad libitum* on different host plants.

period, including the senescent period as well (table 1). Maximum food consumption by the insect was recorded during the I oviposition period for the females and the first 30 days for the males (tables 1 and 2). For instance, *A. crenulata* feeding on *A. hypogaea* consumed 8813 J during I oviposition period out of the total 36241 J for the entire life span; it constitutes 24% of the total food consumed and the males consumed 7114 J (tables 3 and 4). Females of *A. crenulata* consumed more amount of food than the males in all the tested food plants.

With advancing age and increase in body weight, the consumption rate decreased in all the feeding regimes from the I instar until death of the insect. For instance, the feeding rate of *A. crenulata* while feeding on *A. hypogaea* decreased from 15891 J/g/day during the I instar stage to 710 J/g/day at the time of the final oviposition period (figure 2); whereas feeding rate of the male decreased from 15891–552 J. However, the overall feeding rate of female was less than that of the male. Overall feeding rate of the female feeding on *R. communis* leaf was 2405 J/g/day compared to that of 2551 J/g/day for male (table 7).

Corresponding to the increase in food consumption, faeces egested by the insect also increased. For example, faeces egested by a female insect feeding on *R. communis* was 227 J during the I instar and increased to 4501 J at the time of I oviposition period and then decreased to 3139 J during the final oviposition period (table 1).

Table 1. Bioenergetics of female *A. crenulata* feeding *ad libitum* on *R. communis* leaf at 27 ± 1°C, 14:10 L:D, 75% Rh.

Stage	D	C	FU	A	P	R	Ae	Pe_1	Pe_2
Nymph/instar									
I	5·7±0·3	850·91±44·23	227·19±11·92	623·72±33·45	60·67±3·28	563·05±30·36	73·3±3·9	7·62±0·42	9·73±0·54
II	6·2±0·4	1567·85±88·92	465·65±23·39	1102·20±62·27	119·47±6·97	982·73±59·21	70·3±3·9	7·62±0·46	10·84±0·59
III	7·5±0·6	2085·98±123·74	723·84±42·92	1362·14±79·30	165·21±9·86	1196·93±71·24	65·3±3·8	7·92±0·47	12·13±0·68
IV	8·2±0·9	2328·41±144·82	1201·46±78·39	1126·95±79·73	211·42±12·92	915·53±58·88	48·4±3·3	9·08±0·82	18·77±0·92
V	9·0±1·2	2735·48±192·42	1288·41±87·91	1447·07±96·87	339·20±23·06	1107·87±72·97	52·9±3·8	12·14±0·82	23·44±1·28
Adult/oviposition period									
I	19±1·7	9471·00±586·67	4501·58±282·26	4969·42±323·44	927·31±63·57	4042·11±216·26	52·5±3·4	9·79±0·82	18·66±1·31
II	20±2·2	8579·90±584·11	3917·33±280·55	4662·57±329·84	874·11±56·96	3788·46±254·22	54·3±3·7	10·19±0·92	18·74±1·33
III	21±2·6	7262·37±587·95	3380·27±222·13	3882·10±259·95	743·50±50·05	3138·60±203·92	53·5±3·0	10·24±0·84	19·15±1·35
IV	21±3·1	6827·98±513·13	3180·69±208·82	3647·29±225·83	685·73±44·66	2961·56±189·62	53·4±3·7	10·04±0·81	18·80±1·29
V	23±3·6	6621·95±480·84	3170·82±206·16	3451·13±225·83	630·18±43·52	2820·95±171·80	52·1±3·8	9·52±0·79	18·26±1·15
VI	28±6·4	6385·33±464·85	3139·89±198·34	3245·46±212·52	570·56±39·78	2674·90±161·03	50·8±3·8	8·94±0·74	17·58±0·78
Total	168·6	54717·16	25197·13	29520·03	5327·36*	24192·67	53·95	9·74	18·05

*Includes 3490·73 J as egg energy.

Each value (X ± SD) represents the average performance of 5 insects. Values are in J/insect. Efficients are in percentage.

Table 2. Bioenergetics of male *A. crenulata* feeding *ad libitum* on *R. communis* leaf at 27 ± 1°C, 14:10 L:D, 75% Rh.

Stage	D	C	FU	A	P	R	Ae	Pe_1	Pe_2
Nymph/instar									
I	5·7±0·3	850·91±44·23	227·19±11·92	623·72±33·45	60·67±3·28	563·05±30·36	73·3±3·9	7·62±0·42	9·73±0·54
II	6·2±0·4	1567·85±88·92	465·65±23·39	1102·20±62·27	119·47±6·97	982·73±59·21	70·3±3·9	7·62±0·46	10·84±0·59
III	6·7±0·5	1601·03±82·65	600·07±30·91	1000·96±52·54	124·88±7·15	876·08±46·68	62·52±3·19	7·80±0·40	12·47±0·64
IV	7·2±0·9	1859·76±98·87	1015·35±53·89	844·41±43·84	152·50±8·21	691·91±36·86	45·40±2·54	8·21±0·43	18·06±0·96
V	8·1±1·1	2259·67±124·25	1167·18±64·24	1092·49±60·89	231·39±14·09	861·10±47·46	48·35±2·67	10·24±0·57	21·18±1·17
Adult									
I	30±0	7266·43±422·53	3833·67±221·54	3432·66±203·26	203·46±9·86	3229·20±193·76	47·24±3·82	2·80±0·17	5·93±0·36
II	30±0	5394·37±339·82	2890·30±182·09	2504·07±158·78	114·90±7·99	2389·17±152·54	46·42±2·93	2·13±0·14	4·59±0·28
III	19±0	3674·07±238·84	2127·65±137·54	1546·42±108·52	49·60±3·81	1496·82±98·56	42·09±2·74	1·35±0·09	3·21±0·21
Total	112·9	24474·09	12327·06	12147·03	1056·87	11090·16	49·63	4·32	8·70

Each value ($X \pm SD$) represents the average performance of 5 insects. Values are in J/insect. Efficiencies are in percentage.

Table 3. Bioenergetics of female *A. crenulata* feeding *ad libitum* on *A. hypogaea* leaf at 27 ± 1°C. 14:10 L:D, 75% Rh.

Stage	D	C	FU	A	P	R	Ae	Pe_1	Pe_2
Nymph/instar									
I	6·1±0·3	775·51±38·21	209·39±10·24	566·12±27·94	57·62±2·82	508·50±24·97	73·0±3·6	7·43±0·38	10·18±0·50
II	7·3±0·4	1158·67±57·52	347·60±17·42	822·07±41·21	86·90±4·35	735·17±36·96	70·0±3·5	7·50±0·38	10·71±0·54
III	8·2±0·5	1938·40±98·89	693·95±35·42	1244·45±63·47	150·42±7·67	1094·03±56·38	64·2±3·3	7·76±0·39	12·09±0·62
IV	9·1±0·7	2094·89±111·23	978·31±51·84	1116·58±60·28	176·39±9·37	940·19±49·83	53·3±2·8	8·42±0·46	15·80±0·84
V	10·0±1·2	2295·94±126·20	1175·52±64·63	1120·42±62·73	254·39±40·27	866·03±22·40	48·8±2·6	11·08±0·67	22·70±1·27
Adult/oviposition period									
I	20±2·3	8813·02±516·13	1355·19±245·50	4457·83±260·22	726·05±43·19	3731·78±217·89	50·6±3·0	8·24±0·44	16·29±0·93
II	21±2·6	7566·78±457·42	3762·84±228·05	3803·94±252·64	649·56±39·33	3154·38±192·56	50·3±3·1	8·58±0·57	17·08±1·07
III	23±3·1	6754·01±428·51	3400·63±215·86	3353·38±212·85	543·17±34·63	2810·21±178·46	49·6±3·0	8·04±0·45	16·20±1·04
IV	26±5·3	4841·80±325·21	2546·48±166·29	2298·32±151·05	336·37±22·16	1961·95±128·24	47·4±3·1	6·94±0·46	14·64±1·15
Total	130·7	36241·98	17469·97	18772·01	2979·38*	15792·63	51·80	8·22	15·87

*Includes 1667·62 J as egg energy.

Each value (X ± SD) represents the average performance of 5 insects. Values are in J/insect. Efficiencies are in percentage.

Table 4. Bioenergetics of male *A. crenulata* feeding *ad libitum* on *A. hypogaea* leaf at 27 ± 1°C, 14:10 L:D, 75% Rh.

Stage	D	C	FU	A	P	R	Ae	Pe_1	Pe_2
Nymph/instar									
I	6·1±0·3	775·51±38·21	209·39±10·24	566·12±27·94	57·62±2·82	508·50±24·97	73·0±3·6	7·43±0·38	10·18±0·50
II	7·3±0·4	1158·67±57·52	347·60±17·42	822·07±41·21	86·90±4·35	735·17±36·96	70·0±3·5	7·50±0·38	10·71±0·54
III	7·9±0·9	1704·50±91·53	616·69±33·62	1087·81±57·92	128·81±6·99	958·95±51·72	63·8±3·4	7·56±0·42	11·85±0·64
IV	8·7±1·2	2027·02±111·49	921·48±50·71	1105·54±60·79	160·53±8·84	945·60±53·87	54·4±2·62	7·92±0·44	14·52±0·79
V	9·2±1·5	2260·95±129·52	1101·08±62·19	1159·87±65·38	264·79±12·36	945·08±54·69	51·3±2·9	9·50±0·54	18·52±1·04
Adult/month									
I	30±1·0	7113·94±406·88	3941·12±226·48	3172·82±186·58	178·56±10·19	2994·26±171·28	44·6±2·5	2·51±0·15	5·63±0·32
II	29±2·5	4901·52±282·29	2945·81±169·53	1955·71±112·64	64·70±3·75	1891·01±108·98	39·9±2·3	1·32±0·09	3·31±0·21
Total	98·2	19942·11	10083·14	9858·97	941·91	8917·06	49·44	4·72	9·55

Each value (X ± SD) represents the average performance of 5 insects. Values are in J/insect. Efficiencies are in percentage.
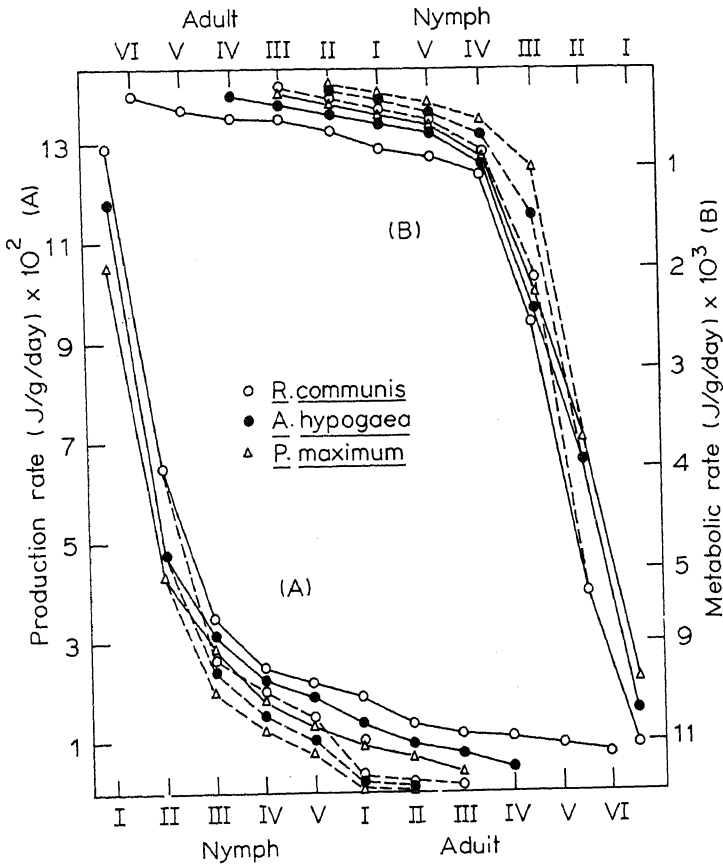
Figure 2. Consumption rate and assimilation rate of *A. crenulata* fed *ad libitum* on different host plants.

Assimilation of energy by the insect was also following a similar trend like that of consumption. For instance, assimilation of a female insect feeding on *P. maximum* was 586 J at the time of I instar and it increased to 4457 J at the time of the I oviposition period. Trends on assimilation rate were also similar to that of the consumption rate (figure 3). For example, the female insect assimilated *P. maximum* at the rate of 10773 J/g/day during the I instar and the rate decreased to 389 J/g/day during final oviposition period. Assimilation efficiency decreased with advancing age. Assimilation efficiency of the insect feeding on *P. maximum* decreased from 72·4% during I instar to 47·4% during the final oviposition period. Major part of the assimilated energy was expended to meet the metabolic requirement of the insect. The female insect feeding on *P. maximum* allocated 24192 J to meet the metabolic requirement from the assimilated energy of 29520 J. Metabolic rate also decreased exponentially with advancing age.

Production of tissue, fat body and egg ultimately increased the energy allocated to production. Females feeding on the tested food plant showed greater increase in production than that of the males (tables 1–6). Production efficiencies increased with advancing age of the nymphal period. However, the adult females feeding on
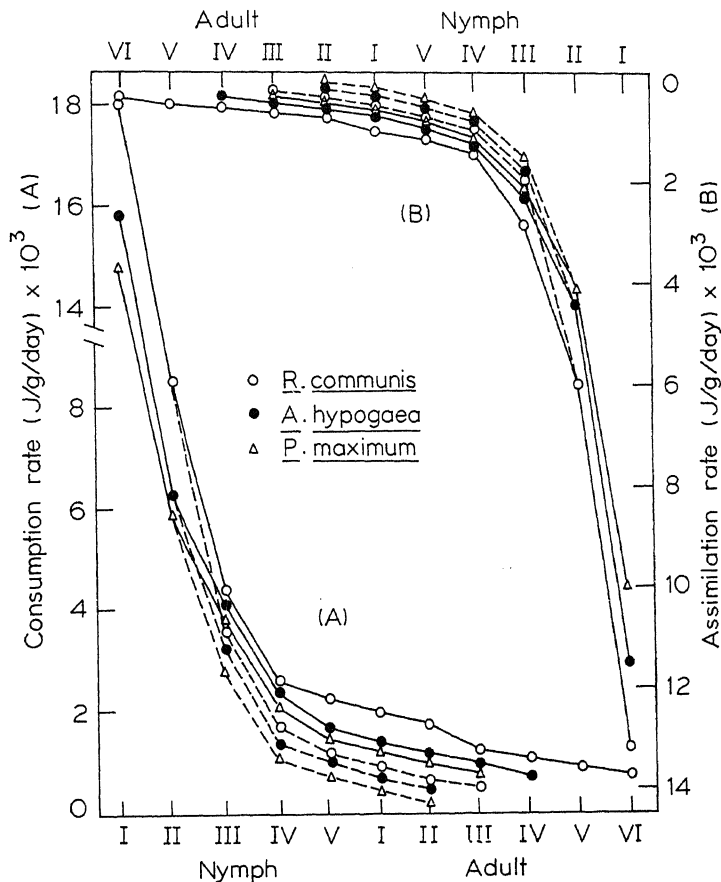
Figure 3. Production and metabolic rate of *A. crenulata* fed *ad libitum* on different host plants.

castor and *A. hypogaea* during the II oviposition period exhibited a moderate increase in the efficiencies of production than during the other periods. For instance, net production efficiency (Pe_2) of females feeding on *R. communis* was 8·7% during I instar and it increased to 23·4% during V instar and subsequently decreased to 16·4 during final oviposition period. *A. crenulata* feeding on castor leaf allocated more amount of assimilated energy to egg production than in the other feeding regimes. Table 8 shows the amount of energy allocated to egg production during the different oviposition period.

4. Discussion

The results presented in this study reveal the following: (i) with advancing age food consumption increases; the other bioenergetics parameters seem to depend on the consumed energy and (ii) energy allocated to egg production increases in the insect fed on the preferred host plant than the one feeding on the alternate hosts. Insects utilize the nutritionally rich host plant more efficiently than the less preferred host

Table 5. Bioenergetics of female *A. crenulata* feeding *ad libitum* on *P. maxicum* leaf at 27 ± 1°C, 14:10 L:D, 75% Rh.

Stage	D	C	FU	A	P	R	Ae	Pe_1	Pe_2
Nymph/instar									
I	6·1±0·5	669·28±36·80	184·72±10·18	484·56±26·68	47·72±2·74	436·84±24·09	72·4±3·9	7·13±0·40	9·85±0·62
II	7·5±0·5	1006·18±59·35	299·84±17·89	706·34±41·72	73·25±4·34	633·01±37·36	70·2±4·2	7·28±0·43	10·37±0·65
III	8·7±1·2	1865·95±113·77	675·47±41·36	1190·48±74·65	138·64±8·67	1051·84±64·27	63·8±3·9	7·43±0·46	11·65±0·71
IV	9·0±1·5	2014·93±128·47	950·03±61·86	1065·90±68·23	160·59±10·39	905·31±60·10	52·9±3·4	7·97±0·51	15·07±0·97
V	9·8±1·9	2113·94±142·39	1097·13±73·58	1016·81±68·14	205·26±14·74	811·55±61·21	48·1±3·3	9·71±0·67	20·19±1·39
Adult/oviposition period									
I	22±4·1	7515·51±524·12	3718·45±260·09	3797·06±272·31	606·88±43·09	3790·18±237·34	50·5±3·5	8·08±0·61	15·98±1·16
II	24±5·7	6719·34±495·07	3371·47±251·32	3347·87±247·78	481·52±36·32	2866·35±214·41	49·8±3·1	7·17±0·62	14·38±1·03
III	25±7·2	3851·58±303·09	2022·93±155·87	1828·65±145·74	215·77±17·76	1612·88±124·31	47·4±3·0	5·60±0·59	11·80±0·98
Total	112·1	25756·71	12320·04	13436·67	1929·63*	11507·04	52·17	7·49	14·36

*Includes 1068·71 J as egg energy.

Each value (X ± SD) represents the average performance of 5 insects. Values are in J/insect. Efficiencies are in percentage.

Table 6. Bioenergetics of male *A. crenulata* feeding *ad libitum* on *P. maxicum* leaf at 27±1°C, 14:10 L:D, 75% Rh.

Stage	D	C	FU	A	P	R	Ae	Pe_1	Pe_2
Nymph/instar									
I	6·1±0·5	669·28±36·80	184·72±10·18	484·56±26·68	47·72±2·74	436·84±24·09	72·4±3·9	7·13±0·40	9·85±0·62
II	7·5±0·5	1006·18±59·35	299·84±17·89	706·34±41·72	73·25±4·34	633·01±37·36	70·2±4·2	7·28±0·43	10·37±0·65
III	8·0±1·0	1219·08±73·14	430·89±25·91	788·19±43·69	90·09±5·48	698·10±42·69	64·6±3·9	7·39±0·45	11·62±0·67
IV	8·7±1·3	1658·40±111·84	794·37±52·69	864·03±56·65	126·37±8·59	737·66±49·56	52·1±3·6	7·63±0·49	14·63±0·96
V	9·3±1·7	2012·36±144·86	1052·46±75·74	959·90±65·97	180·71±12·09	779·19±57·59	47·7±3·58	8·98±0·62	18·83±1·36
Adult/month									
I	30±1·0	5501·21±412·58	3091·68±236·84	2409·53±189·51	136·43±11·25	2273·10±174·59	43·8±3·4	2·48±0·22	5·66±0·49
II	13±3·5	1685·12±106·38	1036·35±63·25	648·77±43·92	20·39±1·09	628·38±47·28	38·5±2·9	1·21±0·10	3·14±0·25
Total	82·6	13751·63	6890·31	6861·32	674·96	6186·36	49·9	4·91	9·84

Each value (X ± SD) represents the average performance of 5 insects. Values are in J/insect. Efficiencies are in percentage.

Table 7. Overall rates of feeding (*Cr*), assimilation (*Ar*), production (*Pr*) and metabolism (*Mr*) of *A. crenulata* feeding on different host plants.

Host plants	Sex	*Cr*	*Ar*	*Pr*	*Mr*
R. communis	Female	2405·29	1270·14	229·22	1040·92
	Male	2551·32	1124·82	97·87	1026·96
A. hypogaea	Female	2405·17	1245·79	199·26	1046·53
	Male	2453·58	1013·00	85·89	927·11
P. maximum	Female	2512·76	1210·85	188·25	1022·60
	Male	2563·38	980·83	77·31	903·52

Table 8. Comparison of food assimilation and egg production of female *A. crenulata* during adult phase.

Food plant	Oviposition–stage	No. of eggs laid	Energy allocated to egg production	Energy assimilated	RE(%)
R. communis	I	58	784·8	4969·02	15·79
	II	52	761·04	4662·57	16·32
	III	43	578·71	3882·10	14·91
	IV	37	494·15	3647·29	13·55
	V	35	459·80	3451·13	13·32
	VI	32	412·23	3245·46	12·70
Total	6	257	3490·73	23857·95	14·63
A. hypogaea	I	42	568·40	4457·83	12·75
	II	38	510·00	3803·94	13·41
	III	32	408·00	3353·38	12·17
	IV	18	182·28	2298·32	7·92
Total	4	110	1668·68	13913·43	11·99
P. maximum	I	37	501·55	3797·06	13·21
	II	30	402·72	3247·87	12·03
	III	12	109·54	1828·65	5·99
Total	3	79	1113·81	8973·58	8·01

plants (Senthamizhselvan 1987). Food consumption of acridids was altered by the presence of both physical and chemical factors (Mulkern 1967; Chapman and Bernays 1977). Orthopterans consume over 60% of their total food consumption during adult phase; it is related to maturation and oviposition, which require a lot of energy (Johannson 1960). *Oryzaephilus surinamensis* is known to consume over 80% of their total food consumption during their adult stage and allocates 42% of its assimilated energy to egg production (White and Sinha 1981). However, *A. crenulata* prefers to feed on nitrogen and water rich leaf with least amount of phenol, when provided with choice of host plant (Sanjayan and Ananthakrishnan 1987). Similar studies were carried out in *Cyrtacanthacris ranaceae* feeding on different host plants by Ananthakrishnan *et al* (1986) who reported a decrease in net production efficiency during III instar and it was due to their estimation of food utilization parameters in 24 h period. This is due to the presence of short-term cellular oscillations and circadian rhythm in feeding activity and different amount of food in gut at different time (Beck 1980; Scriber and Slansky 1981). Assimilation rate of *A. crenulata* depends upon its food consumption rate. Such a positive

Bioenergetics of feeding and metabolic cost of living in freshwater Caridean prawns

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Abstract. The relative partitioning of food energy for processes like moulting, metabolism and growth of the freshwater prawns, *Macrobrachium lanchesteri* (Palaemonidae) and *Caridina weberi* (Atyidae) have been detailed through a bioenergetic scheme. The two species of prawns expend the same amount of food energy for the above processes. Conspecific population density inhibited growth by an overall increase in exuvial production, metabolism and faeces production. Moult-growth relationship of *Macrobrachium lanchesteri* has been presented as a model for determining accurate measurements of moulting and growth of crustaceans.

Keywords. Caridean prawns; food energy; metabolic cost.

1. Introduction

Food energy partitioning has been widely studied in relation to the nutritional score of several animals (Hoar *et al* 1979; Slansky and Scriber 1985; Tytler and Calow 1985; Pandian and Vernberg 1987a, b). Prawns constitute an important aquatic resource for human exploitation and hence, the need for culture fishery of prawns is being stressed the worldover (Pillay 1979; Jhingran 1982). While data on absolute and relative growth in Crustaceans, including prawns, have been detailed by Hartnoll (1982), recent publications have also projected the value of deriving food energy partitioning schemes for Crustaceans (Vernberg 1987). Considerable work on the accelerating effect of eye-stalk ablation on the growth and reproduction of prawns and lobsters is also documented (Mauviot and Castell 1976; Ponnuchamy *et al* 1981; Quackenbush and Herrnkind 1981; Radhakrishnan and Vijayakumaran 1984a, b; Sindhu Kumari and Pandian 1987). Most of the work on freshwater prawns is however restricted to the riverine varieties and paucity of information on the quantification of growth in captivity of these species, leads to inconclusive analyses of their performance in the physiological energetics of food conversion.

The freshwater habitats in and around Bangalore harbour two varieties of prawns, the larger palaemonid *Macrobrachium lanchesteri* (de Man) and the smaller atyids, *Caridina nilotica* (de Man), *C. weberi* and *C. rajadhari* (Anantha Raman *et al* 1986). Earlier, considerable information on the effects of several abiotic and biotic factors on the food conversion and growth of *M. lanchesteri* and *C. weberi* has been reported. Based on these reports, an overview on the feeding energetics and metabolic cost of living of these carideans has been presented in this paper.

2. Material and methods

The details of *M. lanchesteri* and *C. weberi* collection from the local habitats of

Bangalore, methods of laboratory rearing, determination of moulting frequency, food intake, growth and conversion efficiency are detailed by Ponnuchamy *et al* (1983). Table 1 presents the information on the work on physiological energetics of food conversion in *M. lanchesteri* and *C. weberi* in relation to abiotic and biotic factors. The overall data from these works have been used to describe how the energy budget of Caridean prawns are affected by the abiotic and biotic factors.

3. Results and discussion

The food energy budget for a prawn may be represented as follows (see also figure 1):

$$C = L + \Delta G. \tag{1}$$

Where C = food energy consumed; L = Energy loss due to (i) Faeces and urine = Fu, (ii) exuviae = E, (iii) metabolism = M and G = increase or decrease in energy content of prawn.
Therefore

$$C = Fu + E + M + \Delta G. \tag{2}$$

Table 2 summarises the values of Fu, E, M and ΔG calculated as percentages of C elaborated by *M. lanchesteri* reared under various experimental conditions. The best value of G was obtained for the prawn reared in an ideal population density. Conspecific density inhibits G by an overall increase in E, M and Fu. Higher G was also obtained for prawns of smaller sizes and those fed on *Tubifex tubifex* worms. This confirms the size-related growth pattern of the prawn and that tubificid worms provide a good nutritional source for them. A comparatively higher G for a food ration level of 15% again confirms that this level of C is optimum for these prawns. Considerable inhibition of G was evident for large size prawns and for those reared in 6‰ salinity. It is also evident that G was lowered considerably at a ration level of 25%, indicating increased expenditure of energy for M. This is in conformity with the observations of Paloheimo and Dickie (1966a) for fishes. These authors pointed out that a decrease in the gross conversion efficiency of food is

Table 1. Laboratory studies on the effects of abiotic and biotic factors on the food conversion in *M. lanchesteri* and *C. weberi*—data source.

Variables	*M. lanchesteri*	*C. weberi*	References
Population density (no/15 l water)	1, 2, 4, 8, 16 and 24	1, 2, 4, 8, 16 and 24	Ponnuchamy *et al* (1984a)
Body size (mm)	10–20, 21–30, 31–40, 41–45 and 46–50	10–15, 16–20 and 21–25	Ponnuchamy *et al* (1984b)
Quality of food	*Tubifex* worms, Fish muscle	*Tubifex* worms, Fish muscle	Ponnuchamy (1981)
Rational levels (% body weight)	0, 1, 3, 6, 12, 15, 20 and 25	0, 3, 6, 12, 24, 30, 35 and 40	Ponnuchamy *et al* (1983)
Salinity (‰ S)	FW, 2, 4, 6 and 8	FW, 3, 4, 6 and 8	Ponnuchamy (1981)
Light intensity (ft. c.)	6·2, 10·3 and 40·8	6·2, 10·3 and 40·8	Ponnuchamy *et al* (1981)

FW, Freshwater.
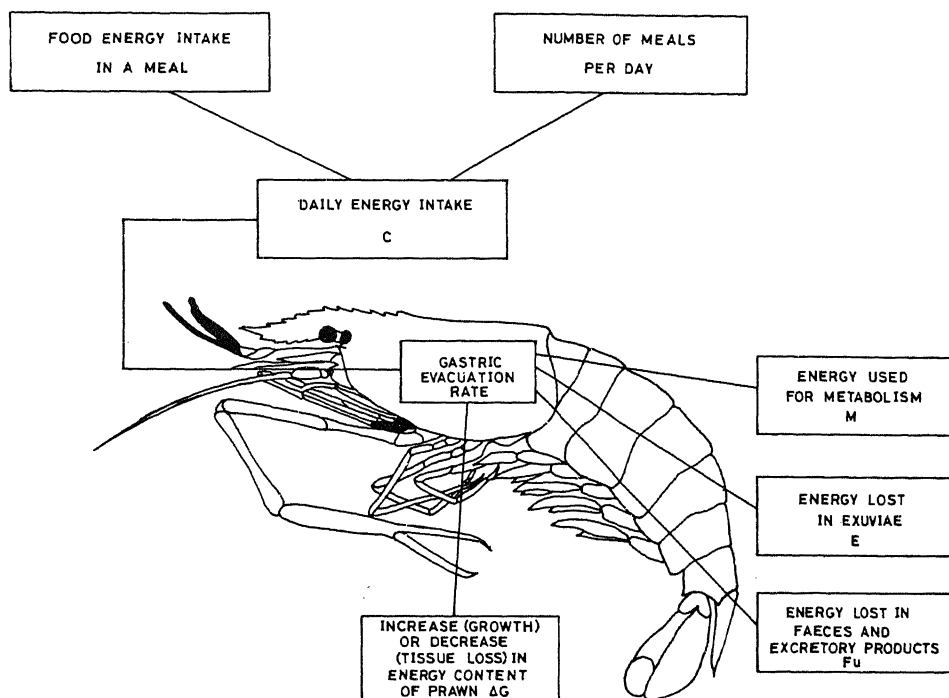
Figure 1. Schematic diagram of food energy partitioning in prawns.

Table 2. Influence of various biotic and abiotic factors on the bioenergetics of feeding of *M. lanchesteri*.

Parameters	mg dry food energy consumed C	Energy conversion as % of C			
		Faeces F	Exuviae E	Metabolism M	Gain G
Population density (4/15 l water)	601·92	41·87	6·60	41·42	10·42
Size					
(10–20 mm)	256·91	27·77	2·32	60·23	9·57
(46–50 mm)	557·17	12·41	1·99	84·76	0·84
Quality of food					
Tubifex worms	539·61	29·53	4·18	61·08	9·38
Fish muscle	307·44	34·29	5·45	56·36	3·89
Ration level					
15%	523·62	39·72	2·48	49·33	8·47
25%	512·99	35·32	2·52	64·48	3·37
Salinity					
6‰	633·98	32·27	3·61	61·27	2·85
Light intensity					
6·2 ft. c.	669·30	14·46	1·37	80·14	4·32
40·8 ft. c.	822·67	14·76	2·13	78·12	4·99
	Mean ± SD	28·24 ± 12·54	3·27 ± 1·68	63·69 ± 13·81	5·81 ± 3·35

dependent on ration size, a relation which could be ascribed to increased metabolic demands associated with increased food processing. During the present study, large size, salinity and increased ration level/day appear to retard the growth of *M. lanchesteri*.

The data on *Fu*, *E*, *M* and *G* as percentages of *C* elaborated by *C. weberi* are indicated in table 3. The best value of *G* was obtained for smaller prawns reared on *Tubifex* worms confirming the size-related growth pattern of the prawns. The next higher value of *G* was observed for those reared on fish muscle, indicating the suitability of the food to elaborate the feeding pattern typical of caridinids (Fryer 1960). The *G* was also comparatively high for the prawns reared on tubificids, 2‰ *S* and light intensity of 10·2 ft.c. Thus, large size, lower rations and higher salinity appear to retard growth of *C. weberi*.

The average partitioning of food energy for *Fu*, *E*, *M* and *G* of *M. lanchesteri* and *C. weberi* under various biotic and abiotic factors are presented in figure 2 (see also tables 2 and 3). In spite of the differences in their finite body size, taxonomic position, food and niche selection (as indicated in table 4), the two natantians apportioned nearly same amount of food energy (*C*) for *Fu*, *E*, *M* and *G*. This again suggests that the two species may not be too diverse in their systematic positions. The average values of food energy partitions for *M. lanchesteri* and *C. weberi* are as follows:
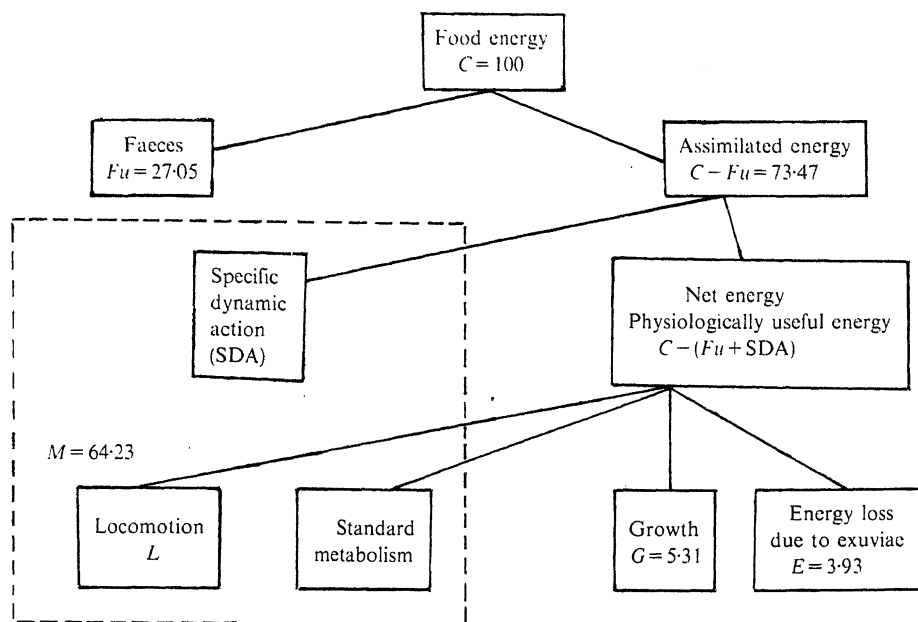
Considerably higher energy expenditure (65%) on metabolism is comparable to the value of 75% reported for other aquatic thermoconformers (Brett 1970). While the *E* of *M. lanchesteri* was lower than *G*, the *E* and *G* of *C. weberi* were equal (see also figure 2). This suggests that moulting is more frequent in *C. weberi* and that the per moult weight (%) is also higher as compared to that in *M. lanchesteri*.

The energy budgets of these two species are not comparable to that reported for

Table 3. Influence of various biotic and abiotic factors on the bioenergetics of feeding of *C. weberi*.

ameters	mg dry food energy consumed C	Energy conversion as % of C			
		Faeces F	Exuviae E	Metabolism M	Gain G
ulation density					
/15 l water)	274·65	34·01	3·78	58·79	3·41
0–15 mm)	185·91	19·08	5·98	64·93	10·01
21–25 mm)	306·62	16·43	5·58	74·41	3·58
lity of food					
ubifex worms	283·11	31·87	3·61	59·07	5·52
ish muscle	513·73	21·33	2·77	69·83	6·06
ion level					
2%	143·17	38·85	7·26	51·90	1·99
0%	368·49	33·13	4·69	57·99	4·19
nity					
‰	283·03	33·30	5·19	55·70	5·81
‰	292·65	36·88	4·56	56·05	2·51
ht intensity					
6·2 ft. c.	429·59	12·32	2·15	81·50	4·53
0·3 ft. c.	283·60	7·02	5·11	82·42	5·45
	Mean ± SD	25·86 ± 10·96	4·60 ± 1·47	64·77 ± 9·97	4·82 ± 2·04
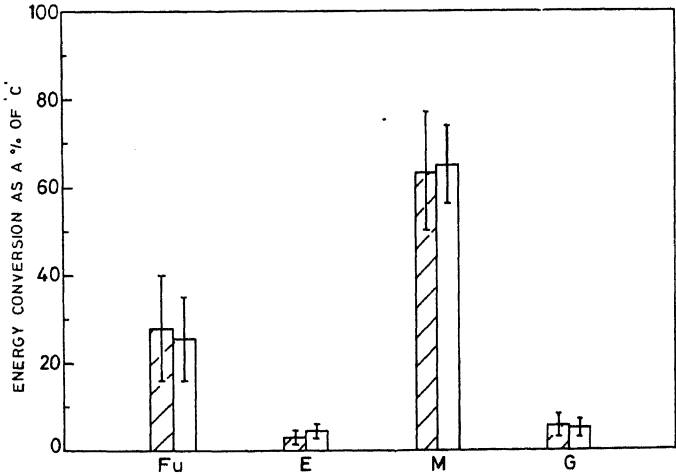
Figure 2. Food energy (*C*) partitioning values for faeces (*Fu*), exuviae (*E*), metabolism (*M*) and growth (*G*) of *M. lanchesteri* (▨) and *C. weberi* (☐).

e marine prawn *Metapenaeus dobsoni* (Thomas 1978: consumption (100%) = assi-
lation (80·18%) + faeces (19·82%); assimilation = growth (38·79%) + moult (0·60%)
metabolism (40·79%)). Markedly higher *G* was reported for *M. dobsoni* as
mpared to that of *M. lanchesteri* or *C. weberi*. Further, lower *E* was observed for

Table 4.

Parameters	*M. lanchesteri*	*C. weberi*
Family	Palaemonidae	Atyidae
Finite body size (mg)	8,050	400
Feeding habit	Carnivore or omnivore	Detritivore or omnivore
Niche	Bottom regions	Marginal zones

M. dobsoni as compared to the two natantians studied presently. Perhaps, the formulated diets offered to *M. dobsoni* yield better growth in that species as compared to the natural foods offered to *M. lanchesteri* and *C. weberi*. Interestingly, all the 3 species of natantians studied so far appear to expend more or less similar amounts of energy on *Fu* which is the second major process of energy loss next to metabolism.

In crustaceans, the growth rate is determined by two processes (i) the increase in size at each moulting and (ii) the frequency with which moults occur (Hepper 1967). Since *M. lanchesteri* and *C. weberi* expend comparable amounts of energy for *Fu*, *E*, *M* and *G*, the former larger species was selected as a model to determine the frequency of moulting. Mauchline (1976) has commented that growth factors (i.e. per moult increments in carapace length) are not constant in many crustaceans, but rather, decrease at successive moults. Hepper (1967) reported that, in lobster, the period between moults increased with age and therefore although the increments would be same at each moult, they would occur less frequently. However, the earlier workers measured the growth indirectly by taking the carapace lengths of the exuviae. Hence, the exact growth may not be correctly represented. Working on the brachyuran, *Gecarcinus lateralis* (Skinner 1966) idealized the sketch of the changes in total biomass as the animal progressed from one intermoult to the next. The author stated that *G* is conservative and a day or so after ecdysis the animal eats the old exoskeleton. In the next intermoult stage, the weight of the animal is greater than its previous intermoult weight, but somewhat less than the maximal weight reached in the premoult period.

, Observation on moult-growth relationship of *M. lanchesteri* are presented in figure 3. The amount of food intake/prawn/day decreased with advance in the weight of the prawns and successive moults. While there were no apparent differences in the food consumption (C), conversion ($Ce^i - Ce^{iv}$), exuvial loss ($E^i - E^{iv}$) and intermoult durations at successive moults the growth/or yield ($Y^i - Y^{iv}$) was slightly sigmoid. Figure 3 conforms to the general observations of Skinner (1966), but a lack of quantified data on food intake, growth and exuvial loss in *Gecarcinus* do not permit a discussion presently.

The relationship between feeding rate, rates of assimilation, metabolism and yield are considered to be important parameters in the culture of aquatic animals (see also Winberg 1960; Paloheimo and Dickie 1965, 1966a, b; Bagenal 1978). Figures 4 and 5 represent these relationships for *M. lanchesteri* and *C. weberi* respectively. In either species, the rates of assimilation and metabolism increased with increases in the feeding rate. Nelson and Knight (1978) working on the juvenile *M. rosenbergii* indicated an apparent increase in the rate of metabolism following ingestion of food. Paloheimo and Dickie (1966a), using an indirect method of assessing total
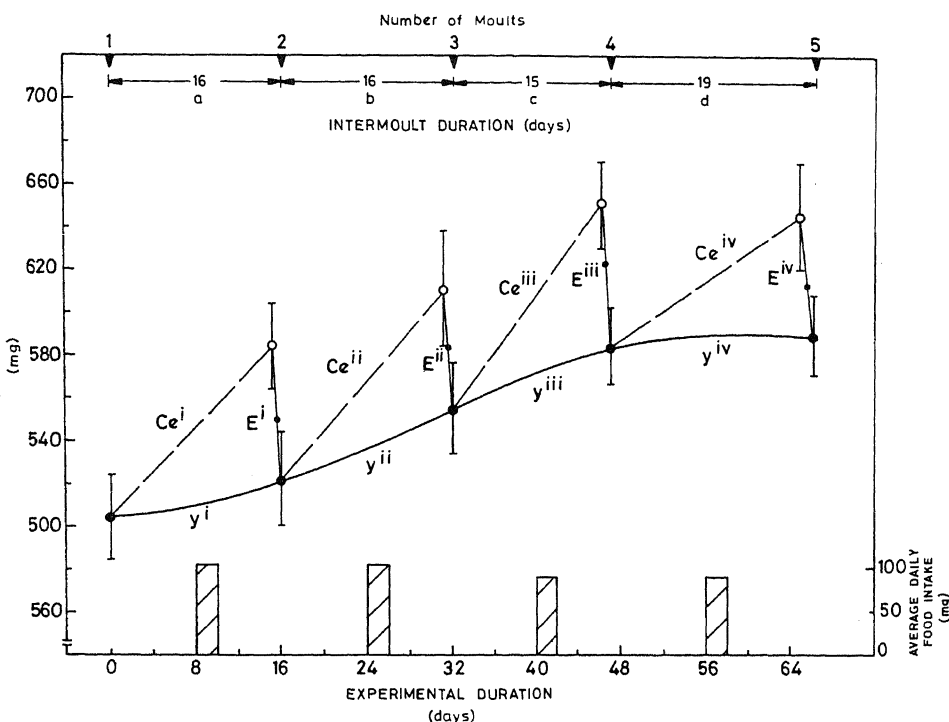
Figure 3. Changes in live body weight of M. lanchesteri in relation to moulting.

Ce^i–Ce^{iv} = Total tissue conversion during the 4 intermoults (a, b, c and d).
E^i–E^{iv} = Tissue loss due to exuviae (2, 3, 4 and 5).
Y^i – Y^{iv} = Actual tissue yield during the 4 intermoults (a, b, c and d).
▨ = Average daily food intake during the 4 intermoults (a, b, c and d).

metabolism, demonstrated that *ad libitum* feeding required an energy expenditure of 6–8 times that for maintenance feeding in fishes. The relationship between the rates of feeding and yield was found to decrease on either side of the optimum feeding level for *M. lanchesteri* (figure 4). However, no such pattern was observed in *C. weberi* and the yield rate did not show any relationship with the feeding rate (figure 5). Under varied experimental conditions, *M. lanchesteri* regulates its yield rate despite increases in feeding rates. In spite of no apparent increases in *G* with increases in feeding rates of the *Poecilia reticulata*, regulation of *B* (= *G*) has also been reported to occur under varied experimental conditions (Krishnamurthy *et al* 1984). From this, it is evident that *M. lanchesteri* would successfully colonize the freshwater habitats and elaborate considerable *G*.

The capacity of *M. lanchesteri* to regulate *G* under various conditions may be the cause for its reported wide distribution in the freshwater habitats (see also Anantha Raman *et al* 1986). Though the size of *M. lanchesteri* is relatively smaller than *M. rosenbergii*, since it spends the entire life in freshwater, it might prove to be more suitable for culture in inland lentic habitats than the latter species.
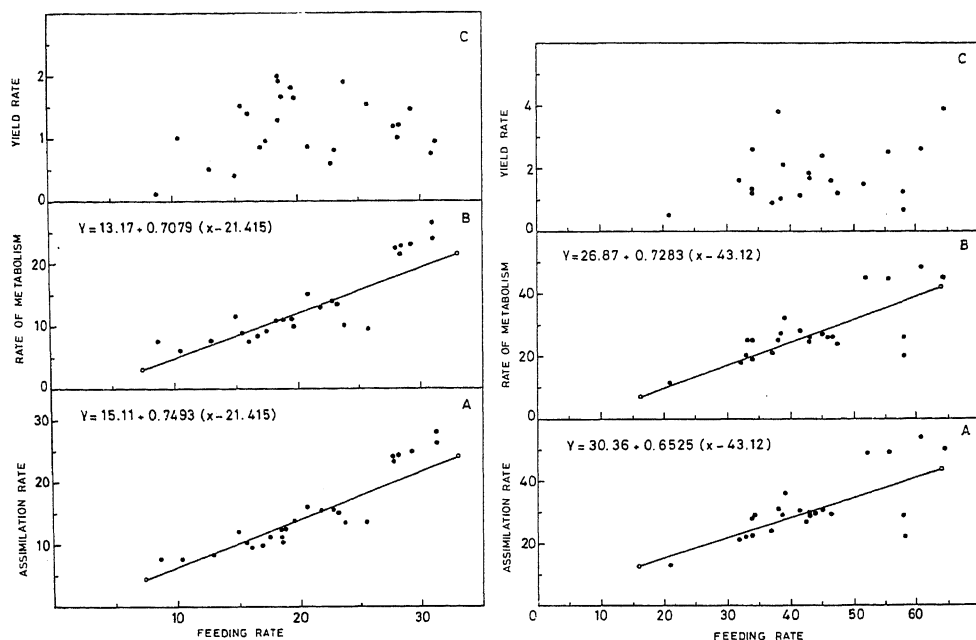
Figures 4 and 5. Scattergrams of rates of assimilation (A), metabolism (B) and yield (C) in relation to the feeding rate of (4) *M. lanchesteri* and (5) *C. weberi*. The points represent the average values obtained for prawns reared under various biotic and abiotic factors.

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Diurnal variation in renal and extra-renal excretion of ammonia-N and urea-N in a freshwater air-breathing teleost, *Heteropneustes fossilis* (Bloch)

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Abstract. Diurnal pattern of excretion of ammonia-N and urea-N through the renal and extra-renal sources were studied in a freshwater air-breathing teleost, *Heteropneustes fossilis* in its aquatic medium. Ammonia-N was the major nitrogen excretory product, however, the rate of excretion of urea-N was comparatively higher than other freshwater teleosts. Almost all ammonia-N (>99%) and about 75% of urea-N were excreted through the extra-renal sources. The excretion rate of urea-N was about 10 times higher than ammonia-N through the renal and ammonia-N was 7–8 times higher than urea-N through extra-renal sources. Existence of a functional ornithine-urea cycle reported in *Heteropneustes fossilis* has been suggested to be the reason for this increased rate of urea excretion.

Nitrogen excretion (total ammonia-N + urea-N) during the night was significantly higher than the day. A higher rate of nitrogen metabolism at night has been suggested for *Heteropneustes fossilis* which has been reported to be nocturnal in habit.

Keywords. Diurnal variation; nitrogen excretion; renal and extra-renal excretion; ammonia-N; urea-N; freshwater air-breathing teleost.

1. Introduction

Freshwater fishes are ammonotelic excreting ammonia as the major nitrogen excretory product primarily through the gills by diffusion (Smith 1929; Forster and Goldstein 1969; Vellas and Serfaty 1974; Walton and Cowey 1982). They are reported to lack a functional ornithine-urea cycle to convert ammonia to urea (Brown and Cohen 1960; Huggins *et al* 1969; Wilson 1973). However, small amounts of urea in different tissues (Holmes and Donaldson 1969) and also as an excretory product (Smith 1929; Brett and Groves 1979; Vellas 1981) have been reported in some freshwater teleosts. It is reported to be produced either from dietary arginine in the presence of arginase (Cvancara 1969a; Saha and Ratha 1987) or through the uricolytic pathway (Goldstein and Forster 1965; Cvancara 1969b; Vellas 1981; Saha and Ratha 1987). Active ureogenesis and ureotelism are characters associated with terrestrial animals where the activity of ornithine-urea cycle enzymes are very high (Cohen 1976). Among freshwater teleosts, there are a few air-breathing species which are capable of surviving temporary water deprivation (Saha 1986) during which ammonia excretion by diffusion through the gills is not possible. Due to their facultative amphibious nature, they are expected to have some special physiological adaptation during their life outside water for detoxification of accumulated ammonia *in vivo*. Saha and Ratha (1987) have reported the

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presence of a complete ornithine-urea cycle with high activity of all the enzymes in the liver and kidney besides a functional uricolytic pathway in one of the freshwater teleosts, *Heteropneustes fossilis*. Having the capacity to convert sufficient amount of ammonia to urea through ornithine-urea cycle, *H. fossilis* should show the nitrogen excretion pattern different from purely aquatic species. This paper presents the findings on the pattern of ammonia-N and urea-N excretion during day and night and through the renal and extra-renal sources in male *H. fossilis*.

2.   Materials and methods

2.1   *Animal*

H. fossilis weighing 30–35 g were purchased from commercial sources in late summer and maintained in plastic aquaria in the laboratory at $20 \pm 20°C$ with 12 h : 12 h light and dark period. Minced pig liver was supplied as food and water was changed on alternate days. The fishes were used after 4 weeks of acclimatization to the laboratory conditions when mortality rate was almost zero and food consumption normal. Food was withheld for 24 h prior to and during the experiment. The experiments were carried out at constant temperature and light conditions to which the fishes are acclimatized.

2.2   *Renal and extra-renal excretion*

Renal and extra-renal excretion were studied in male *H. fossilis* as catheterisation could be successful only in male fish. A 6 cm catheter prepared from polyethylene tubings (0·8 mm diameter) was introduced into the urinary bladder of each fish through the ureter and tied along the copulatory bursa. A balloon was tied at the distal end of the catheter for collection of urine. Each catheterised fish was released at 6 AM in a separate jar containing 1000 ml of bacteria free filtered tap water with 10 mg of streptopenicillin (to stop bacterial growth in the medium). All the jars were covered with bilayers of cheese cloth. The urine was collected after 12 h day and 12 h night at 6 PM and next day at 6 AM respectively by puncturing the distal end of the balloon. The volume of urine collected each time was recorded. The amount of ammonia-N and urea-N excreted through renal sources were estimated in the urine and through extra-renal sources (gills, body surface and faeces) were estimated in the water medium collected at the time of urine collection.

2.3   *Estimations*

2.3a   *Ammonia-N*: Ammonia-N was estimated colorimetrically following the method of Kawakubo *et al* (1983) by collecting it in 0·1 N HCl after isolating from the sample by alkalisation in a suitably modified diffusion bottle. The concentration of ammonia-N was calculated from a linear standard graph prepared following the same method using different concentrations of NH_4Cl.

2.3b   *Urea-N*: Urea-N concentration in the urine and water medium was estimated by converting urea completely to ammonia by urease (Sigma type VI)

prepared in 0·1 M phosphate buffer (pH 7·5) and then estimating the amount of ammonia formed following the above method. The concentration of urea-N was calculated from the difference of ammonia-N concentration: [total ammonia-N (fluid ammonia-N + ammonia-N formed from urea-N)—fluid ammonia-N].

2.4 *Chemicals*

Urease was obtained from Sigma Chemical Co., USA and other chemicals used were of analytical grade. Deionized and double glass distilled ammonia free water was used in all preparations.

3. Results

The major nitrogenous excretory product in *H. fossilis* was found to be ammonia (table 1). It accounted for about 85% of the total ammonia-N and urea-N excreted during 24 h. However, during night total N excretion was significantly higher ($P < 0.05$) than the day. The urine output was also significantly higher ($P < 0.001$) at night than the day. However, the concentration of ammonia-N and urea-N did not differ in the urine produced during the day and the night (table 2).

Almost all ammonia-N ($> 99\%$) and most of urea-N ($\sim 75\%$) were excreted through the extra-renal sources (table 3). However, the renal nitrogen excretion was predominantly of urea-N. The ratio of ammonia-N : urea-N excreted through extra-renal sources ranged between 6·4–8·6 and through renal sources 0·11–0·12. Renal excretion of both ammonia-N ($P < 0.02$) and urea-N ($P < 0.05$) and extra-renal excretion of urea-N ($P < 0.001$) were significantly higher at night than day. Total excretion of ammonia-N did not differ significantly between day and night while the excretion of total urea-N was found to be significantly higher ($P < 0.05$) during the night than day. The ammonia-N : urea-N ratio, thus, decreased from 6·6 during the day to 4·7 at night.

Comparison of data on total ammonia-N and urea-N excreted by various fish species (table 4) indicate that ammonia-N : urea-N excretion ratio is higher in purely

Table 1. Diurnal variation of ammonia-N and urea-N excretion (μg N/g body wt/12 h).

Duration	Ammonia-N	Urea-N	Total ammonia-N + urea-N
12 h day (6 AM–6 PM)	39·5 ± 2·2 (86·7)[a]	6·1 ± 0·5 (13·3)[a]	45·6 ± 2·7 (47·4)[b]
12 h night (6 PM–6 AM)	41·8 ± 3·1 (82·5)[a]	8·9 ± 1·3 (17·5)[a]	50·6 ± 4·5 (52·6)[b]
P (day vs night)	NS	<0·005	<0·05
24 h	78·7 ± 5·8 (85·0)[a]	13·8 ± 2·0 (15·0)[a]	92·5 ± 7·8 (100)[b]

Each value represents mean ± SD from 6 fishes. NS, Not significant.
[a]Per cent of total ammonia-N and urea-N excreted.
[b]Per cent excretion (total-N) during 12 h day, 12 h night and 24 h.

Table 2. Diurnal variation of urine output (ml/g body wt/12 h) and concentration of ammonia-N and urea-N (μg N/ml) in urine.

		Concentration in urine		
	Urine output	Ammonia-N	Urea-N	Ammonia-N/urea-N
12 h day	0·08 ± 0·01	2·2 ± 0·4	18·5 ± 4·2	0·12
12 h night	0·11 ± 0·01	2·4 ± 0·4	20·7 ± 6·4	0·12
		(11%)	(12%)	
P				
(day vs night)	<0·001	NS	NS	

Each value represents mean ± SD from 6 fishes. NS, Not significant.
Per cent increase of concentration are given in parentheses.

ammoniotelic (purely aquatic freshwater) species than other species mentioned which have ureogenic capacity with functional ornithine-urea cycle.

4. Discussion

Although ammonia-N was found to be the major excretory product like other freshwater teleosts, significant amount of urea-N was also being excreted by *H. fossilis* in its normal aquatic medium (table 1). The ornithine-urea cycle was found to be either incomplete or non-functional in all the freshwater teleosts studied by various workers (Brown and Cohen 1960; Huggins *et al* 1969; Wilson 1973). However, a functional ornithine-urea cycle has been reported in 4 (*H. fossilis*, *Clarias batrachus*, *Amphipnous cuchia* and *Anabas testudineus*) out of 5 species of freshwater air-breathing fishes studied (Saha 1986; Saha and Ratha 1987). In all the freshwater and most of the marine fishes excretion of ammonia-N and urea-N ranged between 80–100% and 0–20% respectively (table 4). Gordon *et al* (1969, 1970, 1978) presented a different picture reporting excretion of only about 40% of ammonia-N and 60% urea-N in 3 species of amphibious marine fishes *Periophthalmus sobrinus*, *Sicyasis sanguineus* and *Periophthalmus cantonensis*. However, Morii *et al* (1978) reported 91% excretion of ammonia-N by one of these species, *P. cantonensis* under identical conditions. Inspite of this controversy in some marine fishes, the freshwater fishes show a definite correlation between their ammonia-N excretion and their ability to convert ammonia to urea (table 4). The excretion of ammonia-N was about 88–100% in those species without having a complete or functional ornithine-urea cycle and below 85% in those species with a functional ornithine-urea cycle. In the latter group including *H. fossilis*, some of the ammonia formed possibly got converted to urea and therefore, the excretion of urea-N was relatively higher. The ratio of ammonia-N : urea-N excretion was about 8–10 in the purely aquatic and 4–6 in the amphibious freshwater fishes. The facultative ureogenic amphibious species such as *H. fossilis* having a functional ornithine-urea cycle did behave like an ammoniotelic purely aquatic species by excreting primarily ammonia-N in its normal freshwater habitat (table 1).

Like other freshwater teleosts almost all the ammonia-N (>99%) and most of urea-N (~75%) were excreted through extra-renal sources in *H. fossilis* (table 3). Excretion of N through extra-renal sources as ammonia was reported to be 90% in carp (Smith 1929; Vellas and Serfaty 1974) and 60–90% in coho salmon (McLean

Table 3. Diurnal pattern of renal and extra-renal excretion of ammonia-N and urea-N (μg N/g fish wt/h) in male *H. fossilis*.

Duration	Renal excretion			Extra-renal excretion			Total excretion		
	Ammonia-N	Urea-N	Ammonia-N: Urea-N	Ammonia-N	Urea-N	Ammonia-N: Urea-N	Ammonia-N	Urea-N	Ammonia-N: Urea-N
12 h day	0·015±0·002 (0·6)	0·12±0·02 (24·6)	0·12	3·3±0·18 (99·4)	0·4±0·06 (75·4)	8·6	3·3±0·2	0·50±0·04	6·6
12 h night	0·022±0·005 (0·6)	0·20±0·07 (26·8)	0·11	3·5±0·3 (99·4)	0·54±0·05 (73·2)	6·4	3·5±0·3	0·74±0·11	4·7
P (day vs night)	<0·02	<0·05		NS	<0·001		NS	<0·005	
24 h	0·018±0·003 (0·6)	0·15±0·03 (26·5)	0·12	3·3±0·3 (99·4)	0·42±0·06 (73·5)	7·7	3·3±0·3	0·57±0·08	5·7

Each value represents mean±SD from 6 fishes.
Per cent of nitrogen excretion through renal and extra-renal sources are given in parentheses.
NS, Not significant.

Table 4. Ammonia-N and urea-N excretion by amphibious and fully aquatic fishes immersed in water. Data have been converted into common units (μg N/g body wt/h).

Species	Habitat	Temp (°C)	Ammonia-N	Urea-N	Ammonia-N: Urea-N	References
Amphibious fish						
Periophthalmus sobrinus	Marine	23–30	6·9 (40·6)	10·1 (59·4)	0·68	Gordon *et al* (1969)
Sicyases sanquineus	"	20–23	5·3 (41·4)	7·6 (58·9)	0·70	Gordon *et al* (1970)
Periophthalmus cantonensis	"	20–23	6·3 (46·0)	7·4 (54·0)	0·85	Gordon *et al* (1978)
Periophthalmus cantonensis	"	18–22	4·42 (91·0)	0·44 (9·0)	10·05	Morii *et al* (1978)
Blennius pholis	"	13	1·2 (81·3)	0·3 (18·7)	4·3	Davenport and Sayer (1986)
Channa punctatus	Freshwater	20–22	8·19 (89·1)	1·0 (10·9)	8·19	Roy and Das (1986)
Channa punctatus	"	18–22	4·05 (94·4)	0·24 (5·6)	16·9	Saha (1986)

Anabas testudineus	Freshwater	18–22	5·84 (86·1)	0·94 (13·9)	6·21	Saha (1986)
Clarias batrachus	"	"	5·92 (85·0)	1·04 (15·0)	5·69	"
Amphipnous cuchia	"	"	2·37 (83·7)	0·46 (16·3)	5·15	"
Heteropneustes fossilis	"	"	3·28 (85·0)	0·58 (15·0)	5·75	Present study
Purely aquatic						
Platichthys stellatus	Marine	12	2·40 (88·2)	0·32 (11·8)	7·5	Wood (1958)
Opsanus tau	"	22	3·77 (100)	trace		Read (1971)
Potamotrygon spp.	Freshwater	26–28	4·54 (88·3)	0·62 (11·7)	7·32	Gerst and Thorson (1977)
Cyprinus carpio	"	18·5	4·51 (90·7)	0·46 (9·3)	9·80	Smith (1929)
Cyprinus carpio	"	20	10·73 (88·2)	1·43 (11·8)	7·50	Pequin and Serfaty (1966)

Per cent of excretion are given in parentheses.

and Fraser 1974). In rainbow trout, *Salmo gairdneri* only about 3% of the total N was excreted in the urine and over 60% of the total N excreted was in the form of ammonia (Fromm 1963). Besides the gills, extra-renal excretion of N by the skin has been reported in two mudskipper fishes, *P. cantonensis* and *B. pectinirostris* (Morii *et al* 1978) and an air-breathing marine fish *Blennius pholis* (Davenport and Sayer 1986). The smooth scaleless mucoid skin of *H. fossilis* might have also served as an efficient excretory surface for extra-renal excretion of N along with the gills.

More urine output and excess N excretion in the night by *H. fossilis* (table 2) give an indication of the occurrence of diurnal variability in N excretion in this fish. Circadian variability in N excretion could not be observed in a freshwater sockeye salmon (*Onchorhynchus nerka*) under starved condition (Brett and Zala 1975). We have not come across any report on the circadian pattern of N metabolism or excretion in freshwater air-breathing fishes. King and Goldstein (1983) reported increase in urine flow due to an increased glomerular filtration rate and thus increase in renal ammonia excretion under acidotic condition in gold fish, *Carassius auratus*. Increased metabolism at night might have caused over production of some acidic metabolites resulting in metabolic acidosis in *H. fossilis*. This might have induced significant increase in urine flow and renal excretion of both ammonia-N and urea-N during night than day (table 1). Garg and Sundararaj (1986) suggested that *H. fossilis* was nocturnally active. Enhanced activities of acetylcholinesterase and tyrosine transaminase suggesting higher metabolism during night than day was also reported in *H. fossilis* (Ramanujam *et al* 1981). Renal ammonia-N excretion was very low ($< 1\%$ of total ammonia-N excretion) (table 3) to be considered for any greater physiological significance. However, both renal and extra-renal urea-N excretion were significantly higher during the night than day in *H. fossilis* (table 3). Increased amino acid metabolism might have produced ammonia in excess besides the keto acids resulting into increased urea synthesis by the fish at night.

The results indicate that the freshwater air-breathing teleost, *H. fossilis* does behave like purely aquatic species in excreting primarily ammonia-N as nitrogenous waste in aquatic environment. However, higher quantities of urea-N excretion makes it unique perhaps due to the presence of a functional ornithine-urea cycle, converting some amount of ammonia to urea.

Acknowledgement

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Stylet course of lace bug *Stephanitis typica* (Distant) in coconut leaf

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Abstract. The lace bug *Stephanitis typica* feeds on coconut leaflet from its abaxial side. It inserts its stylet through stomata and sucks the contents of the coconut foliage. Besides this intracellular feeding, the stylet also ruptures the walls of the cells traversed in its course to reach the vascular bundles. The stylet tip in such cases terminates in phloem, thereby suggesting the ability of the bug to acquire the phloem-bound mycoplasma-like organisms, constantly associated with coconut root (wilt) disease in India.

Keywords. Lace bug; *Stephanitis typica*; stylet course; vector; mycoplasma-like organisms; coconut root (wilt) disease.

1. Introduction

Attempts to transmit coconut root (wilt) disease through lace bugs *Stephanitis typica* on the basis of their being the most abundant insect visitors of coconut foliage yielded positive result when they were inoculated on apparently healthy bearing palms in the open field (Nagaraj and Menon 1956; Shanta *et al* 1960) and on coconut seedlings grown in steam-sterilized soil held in cement tubs under an insect-proof house (Shanta *et al* 1964). Solomon *et al* (1983) demonstrated the presence of mycoplasma-like organisms in coconut root (wilt) affected palms and their absence in disease free palms. Since lace bug was abundant on coconut foliage and also it has been incriminated as vector of root (wilt) disease, it was felt that it would be useful to study the stylet course of lace bug in coconut leaf.

2. Materials and methods

2.1 *Fixing feeding insects in situ*

Lace bugs collected from field were enclosed in 15×3 cm test tubes with muslin cloth covered cotton plugs and starved for 16 h. They were then provided with leaflets freshly removed from coconut palm with their distal 30 cm cut and bases dipping in water held in glass jar. Each tube contained about 50 insects. When they settled on the abaxial side of leaflets and started feeding in about half an hour, the leaflets were gently cut in level with the cotton plug taking care not to disturb the feeding bugs. The tube was held in a canister and slowly lowered into a Dewar vessel containing liquid nitrogen and retained there for 2 min. About 30% insects, fixed in the feeding position with their stylets inserted into the lamina (figure 1A) formed the material for study.

2.2 *Transections*

Lace bugs fixed in feeding position by the cold immobilization technique were

transferred along with coconut leaflet bits to phosphate buffered glutaraldehyde fixative (0·05 M phosphate buffer + 2·5% glutaraldehyde + 0·17 M sucrose adjusted to pH 7·4 with NaOH). After 24 h the tissues were washed in buffer and dehydrated in graded alcohol series: 25, 50, 75, 95 and 100% (two changes) for 15 min each, at 4°C. They were further passed through two changes of 100% acetone (30 min each) at room temperature. Infiltration was effected with acetone-resin mixture and in two changes of resin, in a specimen rotator. The specimens were carefully embedded in fresh Spurr's resin and allowed to polymerise at 70°C in an oven for 24 h. Semi thin sections were prepared from the embedments with LKB Ultrotome III. The sections collected over a microscope slide in 10% acetone were dried and stained in methylene blue-Azure II and basic Fuchsin (Humphrey and Pittman 1974).

2.3   *Epidermal peelings*

Peelings of abaxial epidermis were prepared from segments of coconut lamina with the lace bugs killed in feeding position by fixing in 2% glutaraldehyde and paraformaldehyde at 4°C for 24 h. The samples were then cleared by boiling for 10 min in dilute aqueous nitric acid (1:4) containing 2% potassium chlorate. The dermal layers were peeled apart in water with forceps. The mesophyll cells were swept aside with a camel hair brush. Fixed segments were also dechlorophyllated and made transluscent by immersing in boiling lactic acid-phenol-glycerine (2:2:1) for 15 min and subsequently allowed to stay in the clearing agent till the desired degree of clarity was obtained.

3.   **Results and discussion**

Figures 1B and C (epidermal peelings) and figure 1D (transection of coconut leaflet) show that the lace bug inserts its stylet through stomata, which are abundantly present in linear rows on the lower epidermis of coconut leaflet. Based on the absence of any mark of injury to the abaxial epidermis, Mathen *et al* (1979) had suggested stomatal entry of stylet. Johnson (1937) had demonstrated the probe of the tingid *Stephanitis* (*Leptobyrsa*) *rhododendri* Horv. through stomata of rhododendron leaf. Pollard (1959) also upheld the intercellular insertion of stylet by the lace bug *Urentius aegyptiacus* Berg. on egg plant leaf. Comparing the paraffin sections of normal leaflets and those fed by lace bugs, Mathen *et al* (1979) pointed out rupture in the cell walls of mesophyll tissue resulting in cavities immediately inner to the lower epidermis and draining of the contents of palisade cells which, however, retained their shape. They suggested a cell to cell penetration of the stylet before it ended in the vasculature. The depth of stylet penetration is up to 600μm. The maximum thickness of coconut leaflet measured by us is around 400 μm. The insect is therefore theoretically capable of reaching any tissue across the thickness of the coconut leaflet. Figure 2A illustrates the curved stylet track searching for the vascular bundle, breaking the cell walls. The course of the stylet directed straight towards a vascular bundle is illustrated in figure 2B. Each coconut leaf bears several leaflets on either side of a central rachis. Each leaflet has several main and diminutive vascular bundles running parallel with occasional transverse commissures. The smaller bundles are oriented towards abaxial region and the phloem tissues are also in this direction. Access to vascular bundles for the bug is therefore not
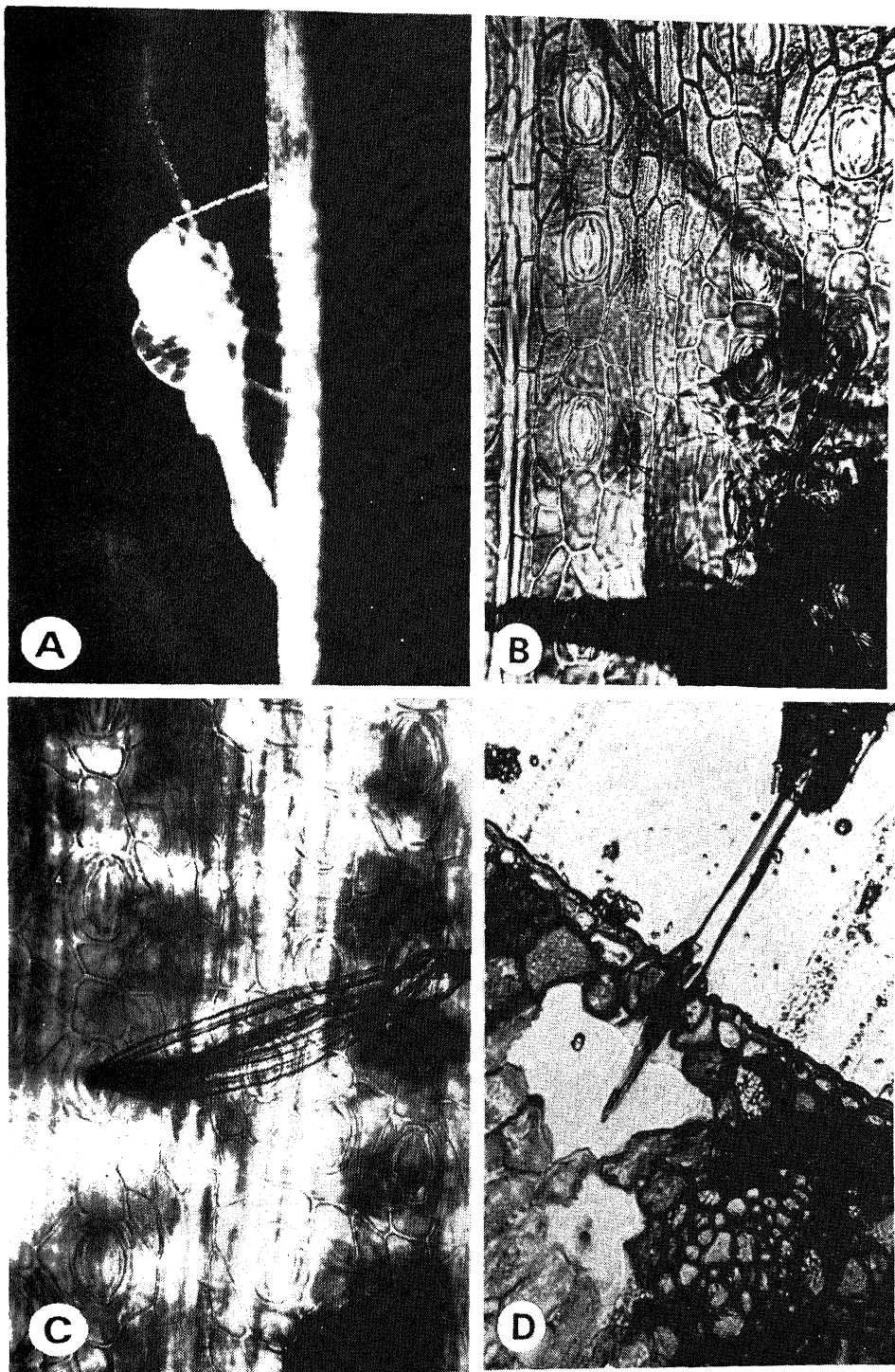
Figure 1. A. *S. typica* in feeding position on coconut leaflet with its stylet inserted (×20). **B and C.** Abaxial epidermal peelings of coconut leaflet showing entry of insect stylet through stoma (**B**, ×320; **C**, ×400). **D.** Transection of coconut leaflet showing insertion of insect stylet through stoma (×400).
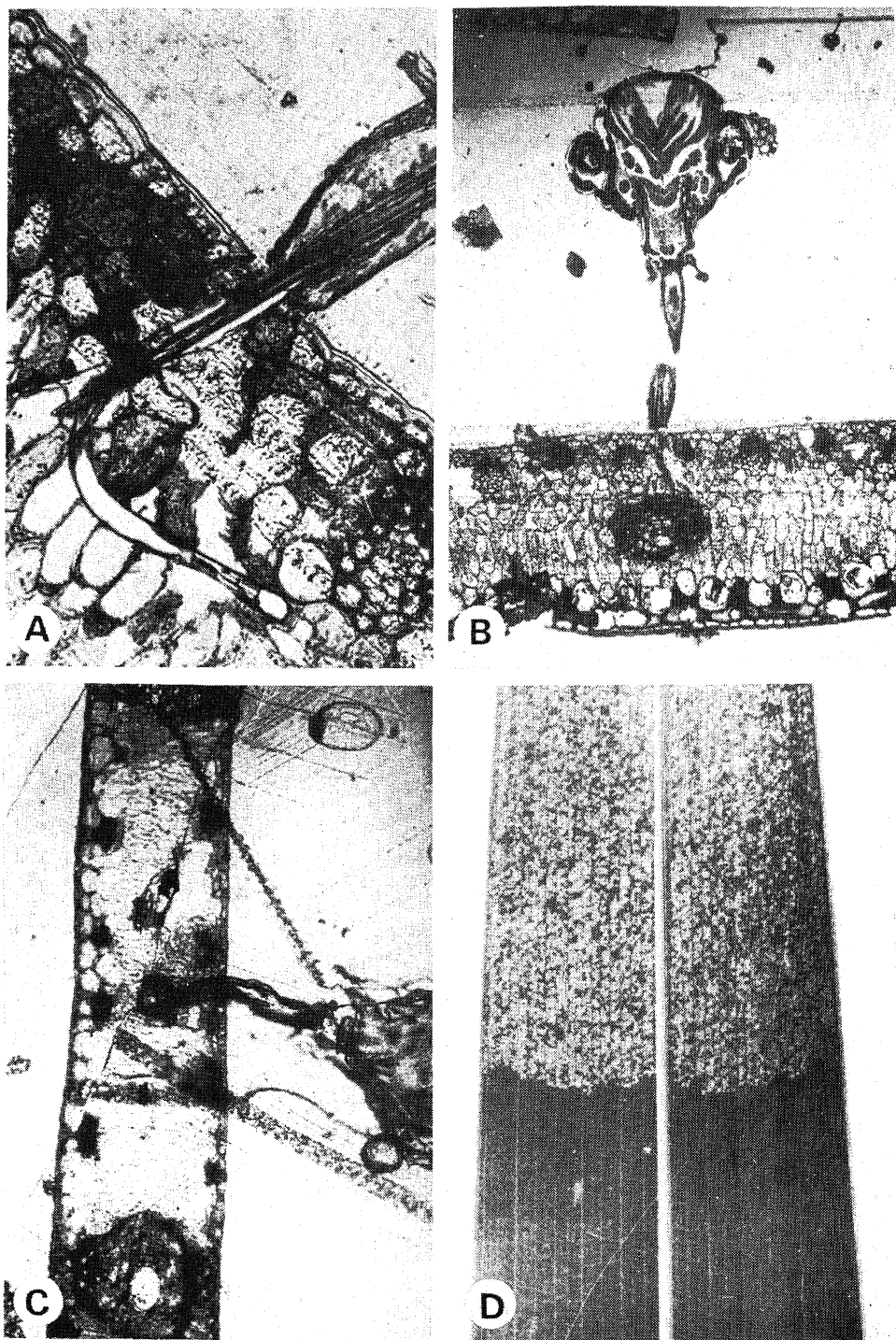
Figure 2. A. Transection of coconut leaflet illustrating the curved course of lace bug stylet through mesophyll to reach vascular bundle (× 420).   **B.** Lace bug stylet directed straight towards a vascular bundle (× 120).   **C.** Termination of stylet in phloem cell in transection of coconut leaflet (× 90).   **D.** Permanent marks produced by the lace bug on the adaxial side of coconut leaflet as a result of feeding from abaxial side (× 1·5).

difficult. The termination of the stylet tip in phloem (figure 2B, C) establishes the ability of the lace bug to reach the tissue. There is thus the possibility that the bug may be able to acquire the phloem-restricted mycoplasma-like organisms. Acquisition of the organisms has been evidenced by the detection of mycoplasma-like organisms in salivary glands of lace bugs examined under electron microscope 18–23 days after they were initially allowed to feed on diseased palm for 5 days and the absence of the organisms in bugs from disease free areas not allowed to feed on diseased palm (Mathen *et al* 1987). The feeding marks left on the adaxial side of the coconut leaflet (figure 2D) suggest that the lace bug does not exclusively feed on phloem, but also feeds on palisade cells. A similar situation is reported in the case of *Piesma quadratum* (Fieb.), which feeds intracellularly on individual parenchyma cells of the leaves of sugar beet, producing visible grey or brown spots but has the stylet terminating in the phloem and hence transmits the phloem-limited leaf curl virus (Proeseler 1980).

Mycoplasma-like organism diseases in plants are transmitted mostly by leaf-hoppers and planthoppers. However, transmission of pear decline by *Psylla pyrisuga* Foester and proliferation disease of carrot by *Trioza nigricornis* Foester (Psyllidae) (Kaloostian 1980); paulownia witches' broom by the stink bug *Halyomorpha mista* Uhler (Pentatomidae) (Shiozawa *et al* 1979); sugar beet savoy disease in north America, now reckoned as of mycoplasma-like organism etiology (Harris 1979) by *Piesma cinereum* (Say) and sugar beet latent rosette disease in German Democratic Republic, caused by rickettsia-like organism confined to phloem cells transmitted by *Piesma quadratum* (Fieb.) (Piesmidae) (Proeseler 1980) gives instances in which Hemiptera other than Auchenorhyncha transmit the mycoplasma-like organisms and rickettsia-like organisms of plant diseases. *Stephanitis typica* (Tingidae), close to Piesmidae in taxonomic status is an addition to the list of bugs other than the conventional groups of leafhoppers and planthoppers as vectors of mycoplasma-like organisms in plants.

Acknowledgements

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Role of chemical components of resistant and susceptible genotypes of cotton and okra in ovipositional preference of cotton leafhopper

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Abstract. Ovipositional preference of cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) on resistant and susceptible genotypes of cotton and okra was found under the influence of chemical components. The non-reducing sugars, tannins, silica and free gossypol in the leaves showed significant and negative correlation with the number of leafhopper eggs.

Keywords. *Amrasca biguttula biguttula*; oviposition; cotton; okra; biochemicals; resistance.

1. Introduction

Cotton leafhopper *Amrasca biguttula biguttula* (Ishida) causes heavy damage by desapping the leaves (Joyce 1961) in cotton and okra in India (Rawat and Sahu 1973; Bhat *et al* 1984), Thailand (Mabbett *et al* 1984) and Pakistan (Ahmad *et al* 1986). There is very little movement of leafhopper nymphs between leaves (Mabbett *et al* 1984) and remain confined to hatching plants. Ovipositing females also ensure the quality of food for proper growth and development of nymphs (Agarwal and Krishnananda 1976; Singh 1985). The differential survival of the pest on resistant and susceptible varieties has been attributed mainly to nutritional and anti-nutritional factors (Uthamasamy 1986; Singh 1987a; Singh 1988) as well as hairiness of leaves (Tidke and Sane 1962; Batra and Gupta 1970). More number of eggs were laid by females on susceptible genotypes as compared to resistant ones in relation to physical characters of leaf (Yadav *et al* 1967; Agarwal and Krishnananda 1976; Khan and Agarwal 1984). Present investigations were carried out to correlate the ovipositional preference of this pest to host suitability in terms of nutritional and antinutritional factors.

2. Materials and methods

Three genotypes each of cotton *Gossypium hirsutum* Linn. viz. BJR-741 (highly resistant), XG-15 USA (moderately resistant) and Acala 4-42 (highly susceptible) and okra viz. *Abelmoschus moschatus* (Linn.) Medic. (highly resistant), Sel-2 (moderately resistant) and AC-302 (highly susceptible) were used at the peak period (August) of leafhopper incidence (Singh 1985).

2.1 *Ovipositional preference*

Thirty leafhopper adults (female: male, 2:1) collected from the susceptible variety of

okra in an aspirator were released in muslin cloth cages enclosing the third expanded leaf from the top in the field. In each genotype, 10 leaves on 10 plants were enclosed and exposed to oviposition for 48 h. Then the leaves were plucked and processed in lectophenol solution (Moffitt and Reynolds 1972) to record the number of eggs laid. The experiment was repeated twice at 3 days interval.

2.2 *Biochemical analyses*

Healthy leaves of each genotype of both crops were used to estimate total sugars (Srinivasan and Bhatia 1953; Yemm and Willis 1954), reducing sugars (Somogyi 1952), free amino acids (Barnett and Naylor 1969; Yemm and Cocking 1955), proteins (AOAC 1970), tannins (Burns 1971) and silica through digestion with triacid mixture. In addition, free gossypol was estimated only in cotton leaves (Mathur *et al* 1972). Fresh leaves were also oven dried at 70°C for 48 h to estimate moisture level. Simple correlations were worked out between the number of eggs laid and amount of various phytochemicals.

3. Results

3.1 *Ovipositional preference*

As evident from the data in table 1, leafhopper females laid significantly more number of eggs in highly susceptible Acala 4–42 (57 eggs/leaf) as compared to highly resistant BJR-741 (14 eggs/leaf) in cotton. Moderately resistant genotype XG-15 USA received intermediate number of eggs (25 eggs/leaf) which was on par with BJR-741. In okra also, highly susceptible AC-302 harboured 162 eggs/leaf which were significantly higher than highly resistant *A. moschatus* (9 eggs/leaf).

3.2 *Biochemical analyses*

Highly susceptible genotype Acala 4–42 of cotton had higher amounts of reducing sugars (2·55%), proteins (18·49%), free amino acids (10·15 mg/g) as compared to highly resistant BJR-741 containing 1·63% reducing sugars, 13·45% proteins and 6 mg/g free amino acids (table 1). In addition, highly susceptible genotype also had significantly lower amounts of antinutritional factors like tannins (15·30 mg/g), free gossypol (6·13 mg/g) and silica (0·7%) as compared to resistant genotypes. In okra also highly susceptible AC-302 contained significantly higher amounts of proteins (26·36%) and free amino acids (8·8 mg/g) and lower amounts of non-reducing (1·94%) and total sugars (2·19%), tannins (13·33 mg/g) and silica (2·1%) as compared to resistant genotypes. Moisture content in both crops did not vary significantly among genotypes.

3.3 *Correlation studies*

Among the principal nutrients (table 2) only non-reducing sugars had significant negative correlation with leafhopper eggs in cotton ($r = -0.72$) and okra

Table 1. Quantitative variations in the phytochemicals in leaves of cotton and okra genotypes in relation to ovipositional preference of leafhopper.

Host	Genotype	Average no. of eggs/leaf	Moisture (%)	Sugars (%)			Protein (%)	Free amino acids (mg/g)	Tannin (mg/g)	Free gossypol (mg/g)	Silica (%)	Rating
				Reducing	Non-reducing	Total						
Cotton	BJR-741	14	71·47	1·63	1·33	2·96	13·45	6·00	23·03	24·01	2·1	HR[a]
	XG-15 USA	25	74·33	1·25	1·27	2·52	16·52	6·83	32·70	36·89	1·7	MR[b]
	Acala 4-42	57	71·55	2·55	0·13	2·68	18·49	10·15	15·30	6·13	0·7	HS[c]
	SE	5·2	NS	0·04	0·09	0·08	0·42	0·25	0·23	0·37	0·07	
	CD (P=0·05)	12·5	—	0·13	0·26	0·24	1·27	0·75	0·70	1·12	0·21	
Okra	A. moschatus	9	80·90	0·22	5·17	5·39	19·80	4·70	32·05	—	6·0	HR[a]
	Sel-2	40	82·48	0·45	3·94	4·39	19·14	4·30	17·45	—	4·3	MR[b]
	AC-302	162	83·27	0·25	1·94	2·19	26·36	8·80	13·33	—	2·1	HS[c]
	SE	10·0	NS	0·01	0·11	0·11	0·36	0·31	0·42		0·14	
	CD (P=0·05)	25·6	—	0·04	0·35	0·34	1·11	0·94	1·29		0·30	

[a]HR, Highly resistant; [b]MR, moderately resistant; [c]HS, highly susceptible.
NS, Not significant.

Table 2. Correlation of leaf phytochemicals with leafhopper eggs in cotton and okra genotypes.

Phytochemical	Cotton		Okra	
	r value	Regression equation	r value	Regression equation
Moisture	0·04	—	0·10	—
Reducing sugars	0·12	—	0·01	—
Non-reducing sugars	−0·72[b]	$Y = 59·29 − 29·96X$	−0·65[a]	$Y = 242·47 − 46·73X$
Total sugars	−0·08	—	−0·25	—
Protein	0·31	—	0·29	—
Free amino acids	0·42	—	0·38	—
Tannins	−0·62[a]	$Y = 72·47 − 1·71X$	−0·71[a]	$Y = 208·85 − 6·64X$
Free gossypol	−0·81[b]	$Y = 57·08 − 1·123X$	—	—
Silica	−0·25	—	−0·63[a]	$Y = 231·96 − 39·10X$

n = 72 (4 independent estimations in each of 3 replications in 6 genotypes).
[a]Significant at 5% level. [b]Significant at 1% level.

($r = −0·65$). Tannin content in cotton ($r = −0·62$) and okra ($r = −0·71$) and free gossypol content in cotton leaves ($r = −0·81$) deterred egg laying showing significant negative correlations. Adverse effects of silica in both crops were also pronounced.

4. Discussion

An array of factors govern the maintenance of insect host plant relations (Kogan 1975). Of these, physical and chemical plant factors play key role in selecting a suitable host plant for oviposition, feeding and shelter (Beck and Schoonhoven 1980; Brewer *et al* 1984). Under caged conditions, leafhopper females exhibited marked difference between nutritionally inferior and superior genotypes for oviposition.

BJR-741 a well known leafhopper resistant variety of cotton (Bhat *et al* 1984) with moderate hair density and hair length (Singh 1985) was less preferred for oviposition because of less percentage of proteins and free amino acids and excess of tannins, free gossypol and silica. Allomonic properties of tannins (Chan *et al* 1978; Sharma *et al* 1982; Singh 1987a, b), gossypol (Sharma and Agarwal 1983; Singh 1987a) and silica (Chakravorty and Sahni 1972; Singh 1987a, b, 1988) against various cotton and okra pests have been well established. Moderately resistant XG-15 USA inspite of poor hair density and hair length on leaf lamina as compared to Acala 4–42 (Singh 1987a) was less preferred for oviposition probably due to high concentrations of allomones like tannins and free gossypol which were even higher than BJR-741.

Among the okra genotypes, *A. moschatus* showing high resistance to leafhopper (Sandhu *et al* 1974; Singh 1988) received less number of eggs which was probably due to higher amounts of non-reducing sugars, tannins and silica. High concentration of non-reducing sugars act as phagodeterrents to sap sucking insects (Nuorteva 1952; Jayaraj 1967; Brewer *et al* 1984). But the highly susceptible AC-302 contained higher amounts of proteins and free amino acids which acted as phagostimulants (Jayaraj 1967; Beck and Schoonhoven 1980) and might have stimulated ovipositional preference.

While comparing the contemporary genotypes of both crops, *A. moschatus* of okra received less number of eggs as compared to BJR-741 of cotton, though the former had poor hair density than the latter (Singh 1985). Ovipositional antixenosis in *A. moschatus* might be due to very high amounts of non-reducing sugars, tannins and silica as compared to BJR-741. Further, lower amounts of non-reducing sugars and silica in BJR-741 appeared to be partly compensated through higher quantity of free gossypol. Comparing the highly susceptible genotypes, AC-302 of okra supported 162 eggs/leaf as compared to Acala 4–42 with 57 eggs/leaf. Besides the presence of more number of leaf-veins suitable for oviposition in AC-302 (Singh 1985), it also had higher percentage of moisture and protein contents as compared to Acala-4–42. It is evident that non-reducing sugars, tannins, free gossypol and silica are the key factors in influencing the ovipositional preference of leafhoppers.

Acknowledgement

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Role of light and temperature in the roosting ecology of tropical microchiropteran bats

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Abstract. Tropical microchiropteran bats need to seek out environments of thermo-neutrality which in the tropics, of course, may mean selecting 'cooler' places for the diurnal roosts. Thus the roosting sites of all the species studied, *Rhinopoma hardwickei, Taphozous melanopogon, Hipposideros speoris, Hipposideros bicolor* and *Megaderma lyra* inhabiting caves, caverns, interiors of temples and a cellar were cooler by several degrees and showed a relatively constant temperature than outside in the open. Such a lower and relatively constant temperature found in the roosts apparently aid in lowering the metabolic rate and cost and may be vital in these tropical species that do not hibernate and some of which may be (such as *Hipposideros speoris*) continuous breeders. This paper presents the temperature and light intensity profiles of bat roosts over 24 h periods for the course of an entire year. It is found that physical protection from predators, relative constancy of temperatures, lower levels of illumination and high humidity seem to determine the choice for roosts in these species of bats.

Keywords. Roosting ecology; behavioural thermoregulation; bats.

1. Introduction

It is well known that the microchiropteran bats are eclectic in their choice of diurnal roosting sites (Brosset 1962). Roosting preferences may involve several factors such as conditioning, habituation and social influence (Klopfer and Hailman 1965). Further, roost preference may not be exercised by merely a visual response to the topology of the substratum but may also involve some secondary, perhaps micro-climatic variables such as temperature, light, humidity, protective cover and shade superimposed on these structures (Schoener 1971). Since the rate of energy expenditure (metabolic rate) is affected by such external factors (Stones and Wiebers 1965) the selection of the appropriate habitat is ecophysiologically important (McNab 1982). This may indicate that microchiropteran bats, owing to the high energy demands, their mode of locomotion and small body-size impose on them, must conserve energy and efficiently cut down on loss of heat to the outer environment.

Tropical microchiropteran bats are successful in solving this ecophysiological problem (Davis 1970). This evolutionary success is reflected in the fact that they roost during the day in many secluded situations from total darkness to broad day light (Greenhall and Paradiso 1968), avoiding high temperatures in the roosts by moving away to cooler parts (Wilson 1971; Wilson and Laval 1974; Vaughan and Vaughan 1986).

If such 'inherent' thermal preferenda exist their role in the habitat selection of the species under natural conditions must be considered. This requires examination of various parameters of roosts. An attempt has been made to study the roosting sites of *Rhinopoma hardwickei, Hipposideros speoris, H. bicolor, Taphozous*

melanopogon and *Megaderma lyra*. A population of the species *R. hardwickei* that inhabits an environment in which thermo-regulatory responses are of particular interest was studied in great detail. Special emphasis was placed on light intensity and temperature vagaries of roosting sites as possible determinative factors in roost selection.

2. Materials and methods

Studies were conducted in various bat roosts from April 1978 through April 1979 in Madurai District (9° 58′ N, 78° 10′ E). Colony morphology and composition are given in table 1.

The climate is that prevailing in tropical plains, consisting of a long, hot and dry summer. Temperature of 37–40°C may prevail for several days. Precipitation occurs primarily during September through December. The most striking feature is that these months may pass with little or no rain (Meher–Homji 1978). Temperature and humidity in the roosts were recorded with automatic Lambrecht KG, Goettingen Type 252 UA, thermohygrographs. Special temperature probes were used as necessary.

Light intensity profiles in the roosting areas were measured using an AEG lux meter and a UDT (40 lux) optometer. Light intensity profiles were obtained from dawn to dusk as measured in the region of the caves where the first cluster of bats are found. Their general behaviour were studied using a noctovision sniperscope (home made). Meteorological data (wind speed, wind direction, precipitation, ambient temperature and humidity) were obtained from the Meteorological station of the Department of Animal behaviour, School of Biological Sciences, Madurai Kamaraj University, Madurai.

3. Results

The mean ambient air temperature and humidity outside the roosts fluctuated widely from 20·6–36·4°C and 25–95% respectively during the study period (figure 1A), while the roosts showed relatively constant temperature. The patterns of temperature change in the roosting sites of study subjects are given in figures 1 and 2.

The constancy of temperature was well pronounced in those caves inhabited by *H. speoris* and *H. bicolor*, at KKK (figures 1B and 2), where the absolute temperature is much lower and more constant than the ambient temperature. Over an year the mean recorded temperature in those roosts was 27°C. However, at KKB and KHC where *H. speoris* roosts, the temperature was higher at a relative constancy of 30°C (figure 2).

Caves occupied by *M. lyra* showed temperature variations of 1·5–2°C at SLP and PM whereas the range of variations exceeded approximately to 7°C at KKK (figures 1 and 2). Of particular interest was the maximal temperature fluctuations of the diurnal roost of *M. lyra* which occurred during the months of April and November. Typically on 16 April 1978 the highest temperature recorded was 30·5°C at mid-day at SLP and the lowest temperature for the rest of the day was 30°C.

In crevices inhabited by *T. melanopogon* the temperature variations were moderate, ranging from 5–6°C during the months of March through December in contrast to the much more marked variations of the ambient temperature outside

Table 1. Population size (approximate) of the various species and a brief description of their roosting sites.

Location	Species	Population		Nature of roosting site
Keela Kuyil Kudi (KKK)	*Hipposideros speoris*	ca. 500–600	Cave	Dark: temperature and humidity constant
	Hipposideros bicolor	ca. 150	Cave	-"-
	Hipposideros speoris	ca. 50	Cave	-"-
	Megaderma lyra	ca. 20–30	Cave	Well lit and temperature constant
	Taphozous melanopogon	ca. 70	Creek	-"-
Kanavai Katha Bootham (KKB)	*Rhinopoma hardwickei*	ca. 1500	Cavern	-"-
	Hipposideros speoris	ca. 1000	Cave	Dark: polluted by NH_3, temperature constant
Pulian Kulam (PKM)	-"-	ca. 100	Crevice	Well lit and temperature constant
Kennet Hospital Cellar (KHC)	-"-	ca. 30	Cellar	Dimly lit and temperature constant
Pannian Malai (PM)	*Megaderma lyra*	ca. 200	Cave	Dark and temperature constant
	Hipposideros speoris	ca. 400		
	Rhinopoma hardwickei	ca. 400		
Seelayampatti (SLP)	*Megaderma lyra*	ca. 20	Temple roost	Well lit and temperature constant
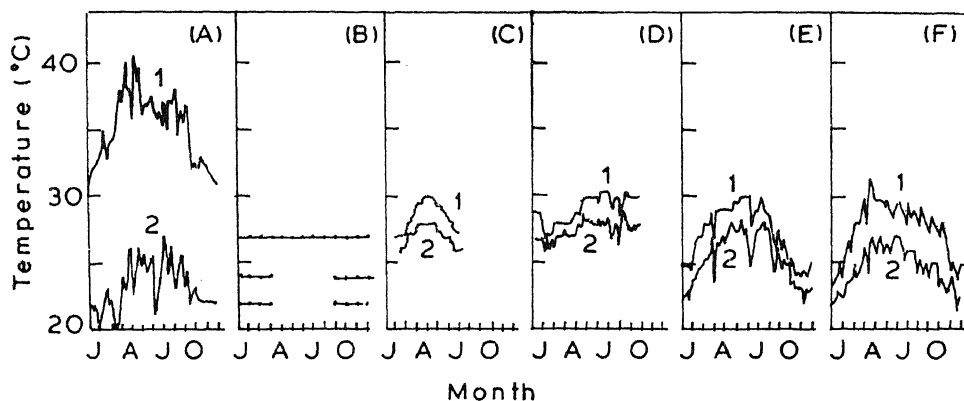
Figure 1. Patterns of ambient temperature recorded at the meteorological station with a thermograph and at various roosting sites inhabited by different species of bats for a period of one year (1978–1979). Maximum and minimum temperatures are shown as continuous and broken lines respectively. **A.** Maximum and minimum temperatures of the outside environment. Temperatures at the roosting sites inhabited by; **B.** *H. speoris* at KKK (●) and *M. lyra* at PM (-●-); **C.** *H. speoris* at KHC; **D.** *R. hardwickei* at KKB; **E.** *M. lyra* at KKK; **F.** *T. melanopogon* at KKK.

approximately upto 13°C. Over a 24 h period the temperature within the crevice showed only minor variations which ranged from 2–3°C.

R. hardwickei preferred a different type of roost. Even during the hot summer days, the mean temperature variation was only 2·5°C at the roost. These animals moved daily deeper inside the cave or crevice from the original roosting sites and selected areas where temperature fluctuations were insignificant. For example on 21 March 1979 the temperature at the mouth of the crevice ranged from 26–32°C (figure 3) but the magnitude of temperature variation was less at a place 5·5 m interior. The temperature at the deepest part of the crevice, which is approximately 14·5 m from the entrance where the bats were found most of the day, was between 27 and 29°C. The bats progressively moved to cooler places, thus covering various areas in the crevice in the course of the day (figure 3).

Light intensity profiles are shown in figure 4 for all the roosting areas. The caves occupied by *T. melanopogon*, *R. hardwickei* and *M. lyra* are well lit during the day light hours, the intensity of light varying between 0·1 and 115 lux, 0·3 and 24 lux and 0·05 and 0·6 lux respectively. A cave that is occupied by *H. speoris* at KKK characterized absolute darkness (1000 s exposure of the photo element registered no light at all). However, caves such as the PKM occupied by the same species is well lit throughout the day. The variations recorded from dawn to dusk ranged between 1·3 and 18 lux.

4. Discussion

The study reveals that most species of bats occupy regions of the roosts, where temperature fluctuation is relatively insignificant (figures 1–3). Similar temperature preference by pallid bats is known to some extent (Vaughan and O'Shea 1976). Vaughan and O'Shea (1976) recorded the temperature in a grotto which varied by
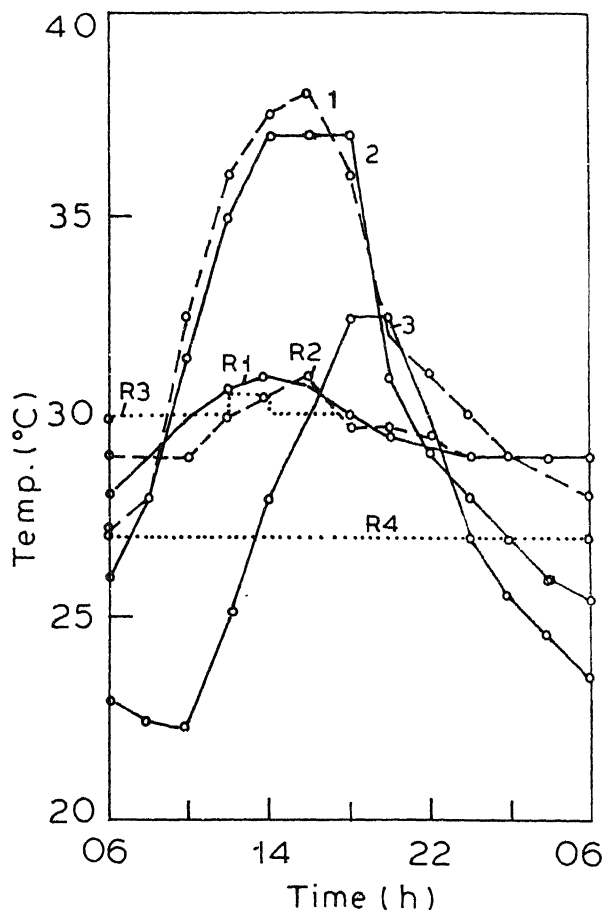
Figure 2. Patterns of ambient temperature recorded with a thermograph at the meteorological station on February 28(3), April 15(2) and April 16(1). R_1-daily profile of temperature of the cave inhabited by *H. speoris* at PKM; R_2-daily profile of temperature of the roosting site inhabited by *H. speoris* at KKB; R_3-daily profile of temperature of the roosting site inhabited by *M. lyra* at SLP; R_4-daily profile of temperature of the cave inhabited by *H. bicolor* at KKK.

y 0·8°C over a 24 h cycle. In addition to this favourable stable temperature lid bats cluster and as a result decrease the total surface area exposed per bat ughan and O'Shea 1976). The metabolic cost is greatly reduced by formation of ge colonies and clustering behaviour and choice of roost configuration that ximise retention of dissipated heat (Tuttle 1975). Such clustering was not noticed ong the bats studied by me. If the roosting microclimate is affected, only the ernate roosting sites can afford better protection as reported for *Myotis sodalis* Humphrey *et al* (1977) and the African yellow winged bat *Lavia frons* (Vaughan d Vaughan 1986). For instance the relative scarcity of free-tailed bats *Tadarida* cies (Davis *et al* 1962) where the temperature is above 30°C suggest the currence of some temperature selection. Similar roosting site preference as per the vironmental factors is reported in Jamaican bats also (Goodwin 1970). Even ugh the different roosting areas of *H. speoris* have different roost temperature,
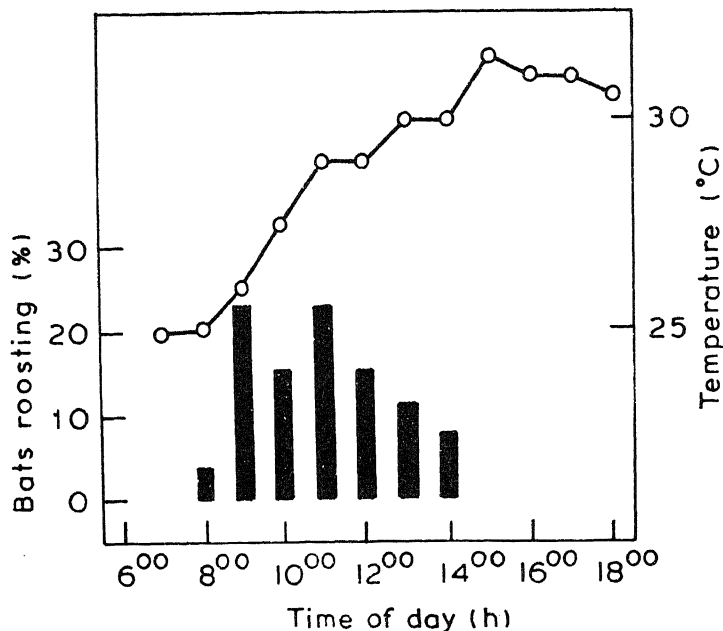
Figure 3. The line shows increase in the temperature 2 meters into the cave during daylight hours. The histogram denotes the number of bats roosting around the spot where the thermograph was placed. Note that temperature increased from 25–32°C and movement of bats to deeper parts of the caves is reflected in declining numbers where the thermograph was located.

the relative constancy of temperature is significant. This condition of thermal neutrality promotes the maintenance of homeothermy and rapid growth in the young (Kunz 1973). The thermal neutrality is particularly important for some of the tropical bats are continuous breeders.

Marked temperature fluctuations are not suitable for the roosts (O'Farrell and Bradley 1970). In some instances temperatures within the caves may vary greatly over the seasons and depending upon the patterns of air circulation within the caves. In such a situation, bats possibly move to cooler sites in the roosts at times of 'heat stress'. In roost flying also increases the surface area for heat loss and the bats seem to be able to reduce their body temperature (Herreid 1963). Bats prefer a constant temperature surrounding even at the expense of their metabolic energy. So it is not surprising to find that cave bats select temperature constancy even within the cave system. Some bats in the day roosts, for e.g. *T. melanopogon* and more particularly *R. hardwickei* are able to reduce the environmental stress by behavioural means like selecting the areas of the caves where the temperature does not fluctuate significantly.

Such diurnal thermo-regulatory movements have also been described in some insectivorous bats (Licht and Leitner 1967). These intra-roost migrations form a compromise between avoidance of fluctuating temperature and protected roosts. Downward movement from the roof of the cave and progress into the interior recesses of the cave when the sun is in its zenith are common and regular occurrence among the bats studied. They remained as one group and 'cooling off'
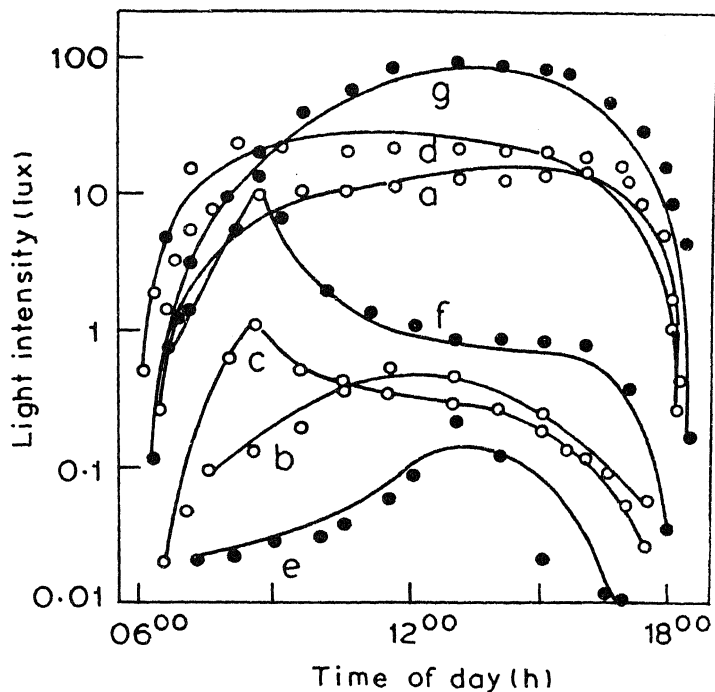
Figure 4. Variations in the light intensity of various roosting sites. **a.** *R. hardwickei* at KKB; **b.** *H. speoris* at KKB; **c.** *H. speoris* at KHC; **d.** *H. speoris* at PKM; **e.** *H. speoris*; **f.** *M. lyra* at KKK; **g.** *T. melanopogon* at KKK.

flights were very meagre. This is in full agreement with the view of Studier and O'Farrell (1972) that behavioural thermoregulation by 'in-roost movements' is as important as physiological thermoregulation in providing these bats a survival value. Such downward movement to the floor and breaking of tight clusters are reported in *Myotis nigricans* (Wilson 1971; Wilson and Laval 1974).

A high degree of humidity is required by tropical bats (Brosset 1962) but not essential for specialised functions as hibernation (Fenton 1970). Temperature seems to be a prime factor in the roosting ecology of bats since *R. hardwickei* and *T. melanopogon* live under relatively less humid regions. These bats can also be observed in well lit places, while *H. speoris* are found in total darkness in diurnal haunts (figure 4). Darkness is no doubt a favourable factor because of the protection from predation. Kunz (1973) even reported that at Wilmore, bats occupied dimly lighted areas and the darkest areas were not occupied. Tropical bats, however, do roost in the darkest places of the caves provided they have access to relative temperature constancy.

Both rapid dissipation of body heat in the morning prior to torpor through most of the day and passive warming in the evening prior to taking to flight depend upon the ability of the bats to position themselves appropriately in the thermal gradient in roosts (Studier and O'Farrell 1972). This is also the case of tropical bats where warming up prior to flight is achieved by moving to the proximity of the cave mouth. It serves dual purpose of warming up the body and 'light sampling' (Twente 1955) without departing from the roosts (Kunz 1974). Interestingly *H. speoris* which

have colonized in Madurai cave environments where the temperature is virtually invariant also do not move about in the cave. In fact, these bats show a remarkable sense of 'personal space' and site fidelity and roost in them day after day (Selvanayagam and Marimuthu 1984). *R. hardwickei* as noted earlier resorts to behavioural thermoregulation to some degree and as a consequence denies itself a right to place fidelity and personal space.

Acknowledgements

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Growth in the Asian elephant

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Abstract. Records of captive Asian elephants (*Elephas maximus*) were used to derive parameters of the von Bertalanffy function for growth in height, body weight and circumference of tusks with age. There was some evidence for a post-pubertal secondary growth spurt in both male and female elephants. Domestic elephants which were born in captivity or captured at a young age also showed a reduced growth in height in both the sexes and in body weight in males compared to wild elephants. Aspects of allometric growth such as height-body weight relationship are examined. The height was twice the circumference of front foot throughout the life span, indicating an isometric relationship.

Keywords. Asian elephant; *Elephas maximus*; growth; von Bertalanffy function.

1. Introduction

Aspects of growth in the Asian elephant *Elephas maximus* (Burne 1943; Rensch and Harde 1955; McKay 1973; Kurt 1974), have not been examined with the same rigour as in the African elephant *Loxodonta africana* (Hanks 1972; Laws *et al* 1975). This is ironical since a lot of data are available for Asian elephants in captivity. The present study was carried out to derive equations for growth in body length (in this case, the height at withers or shoulder height), body weight and tusk circumference at lip line with age, and also examine aspects of allometric growth. These relationships could be profitably used in various ecological studies, which may involve ageing wild elephants or estimating biomass of the population.

2. Methods

Hanks (1972) and Laws *et al* (1975) derived growth equations based on von Bertalanffy functions (von Bertalanffy 1938; Beverton and Holt 1957) for the African elephant. Their data base came from large samples of culled elephants, which were aged from their dentition (Laws 1966) and various body measurements. These studies were cross-sectional; that is, for each elephant the height or weight was known at only one instant in time. On the other hand, we collected data on elephants born in captivity or captured from the wild, which were kept for varying periods of time and measurements, particularly of shoulder height, taken every year. For some elephants records of growth were available for 40–50 successive years. All the elephants were captured in southern India and maintained by Forest Departments under semi-natural condition (in all cases inside the forest habitat itself, as opposed to those kept in temples in the towns). Records of nearly all elephants born in captivity (n = 165) or captured (n = 525) in the Madras Presidency and Tamil Nadu state during 1926–1984 were obtained. Some records (n = 74) also pertained

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to those captured during 1885–1925. Information on elephants born in captivity (n = 24) or captured (n = 146) in the Karnataka state was also obtained.

Most of the records pertain to only the shoulder height of elephants; much less information was available on circumference of front foot. Data on body weights have been recorded only in domestic elephants kept in the Mudumalai Sanctuary, where they have been weighed on a heavy-duty weighbridge from 1973 onwards. Practically no data were available on tusk growth. In Mudumalai Sanctuary 20–25 elephants were measured during 4 successive years (1981–1984). A number of elephant camps in Karnataka were visited during 1983 and measurements on 40 elephants were recorded. Measurement of tusks were also included in addition to height and circumference of front foot.

2.1 *Selection of elephants for use in the analysis*

For elephants born in captivity the available data could be used without any problem if their dates of birth were known. Captured elephants had to be aged as accurately as possible before the data could be included. We selected elephants for use in the analysis by the following procedure.

(i) Using the data on elephants born in captivity, von Bertalanffy growth equations were generated seperately for males and females aged 0–15 years. Since most of the elephants born in captivity were sold at an early age, the records were available for a sufficient number of them only up to age 15 years.

(ii) Of the elephants captured, only those kept in captivity for 5 or more years were selected. From this list, male elephants measuring below 205 cm (age 8 years) and female elephants below 198 cm (age 10 years) at the time of capture were reassigned ages based on the von Bertalanffy equations derived for elephants born in captivity. This reassignment was necessary since the age fixed at capture by the attending veterinarian would have been subjective. We noticed a distinct tendency to over-estimate the age at capture. Records of elephants above these heights were eliminated since accurate ageing was not possible.

(iii) The data from elephants born in captivity and those reassigned ages were pooled together for further analysis on growth in height. Any slight inaccuracy in ageing captured elephants was unlikely to influence the final results since data on growth were included for at least 5 years (records for many of them were available for over 40 years). This was confirmed by a check later.

(iv) In addition, the heights of many wild adult elephants, which could be expected to have attained their maximum height, were determined by a photographic method described elsewhere (Douglas-Hamilton 1972; Jachmann 1980; Sukumar 1985).

2.2 *von Bertalanffy functions*

The functions derived by von Bertalanffy (1938) have been found suitable for studies on growth phenomena in vertebrates including fishes (Beverton and Holt 1957) and elephants (Hanks 1972; Laws *et al* 1975). The general form of the equation used is

$$S_t = S_\infty \{1 - \exp[-K(t - t_0)]\}^M,$$

where S_t = size (height, weight, etc.) at age t; S_∞ = asymptotic size; K = coefficient of

catabolism, a fitted constant; t = age of animal (years); t_0 = theoretical age at which the animal would have zero size (this constant is usually artificial) and M = power of the function.

For growth in body weight the cubic form of the above equation can be used:

$$W_t = W_\infty \{1 - \exp[-K(t - t_0)]\}^3.$$

For growth in body length or height the equation used is:

$$L_t = L_\infty \{1 - \exp[-K(t - t_0)]\}.$$

2.3 *Computer analysis*

The parameters S_∞, K and t_0 of the von Bertalanffy function were determined using the non-linear least square method by an iterative procedure. For a set of starting values of these parameters, the sum of the squares of deviation between expected and observed size was computed, and also the derivative of this quantity with respect to S_∞, K and t_0. The Fletcher-Powel algorithm was used to modify the values of S_∞, K and t_0 at each stage, such that the sum of squares of deviates was minimized. The subroutine FMFP of the Scientific Subroutine Package developed by IBM was used for this purpose on a DEC 1090 computer at the Indian Institute of Science. The sensitivity of the method was tested by using different sets of initial values. It was seen that they all converged to the same final values of the parameters. A further check was carried out by the conjugate gradient method of minimization (subroutine FMCG of IBM SSP routine) which also yielded very similar results.

3. Results and Discussion

The results of all the growth equations presented here are based on the data set combining elephants born in captivity and those captured from the wild.

3.1 *Growth in height*

3.1.1 *Male elephants*: For male elephants the equation derived for age 0–15 years (507 measurements of 118 elephants) was

$$H_t = 236 \{1 - \exp[-0.182(t + 2.82)]\} \text{ cm.}$$

This gave a good fit to the data up to 15 years. But the observed heights of adult bulls above 15 years are much greater than the heights predicted by this equation. This suggests that a secondary growth spurt associated with puberty occurs in bulls, similar to that observed in African elephants by the same procedure (Laws *et al* 1975). Hanks (1972), however, did not find any evidence for a secondary growth spurt in the African elephant.

Another set of parameters was derived for bulls aged 15–60 years (321 measurements, 34 elephants), which gave a good fit for this age range

$$H_t = 259 \{1 - \exp[-0.124(t + 2.84)]\} \text{ cm.}$$

A third equation was derived for bulls using the combined data for 0–60 years (816 measurements, 119 elephants), which gave a good fit only above 3 years (figure 1)

$$H_t = 256\{1 - \exp[-0.133(t + 3.58)]\}.$$

3.1.2 Female elephants:

3.1.2 *Female elephants*: The equation fitted to data for age 0–15 years (376 measurements, 90 elephants) was

$$H_t = 215\{1 - \exp[-0.193(t + 3.02)]\} \text{ cm}.$$

Once again the asymptotic height of 215 cm is much lower than the average maximum height attained by captive elephants. This suggests a secondary growth spurt in female Asian elephants. Laws *et al* (1975) did not observe such a growth spurt in female African elephants. This phenomenon has to be examined in more detail. Anyhow, a second equation was fitted for females aged 15–70 years (330
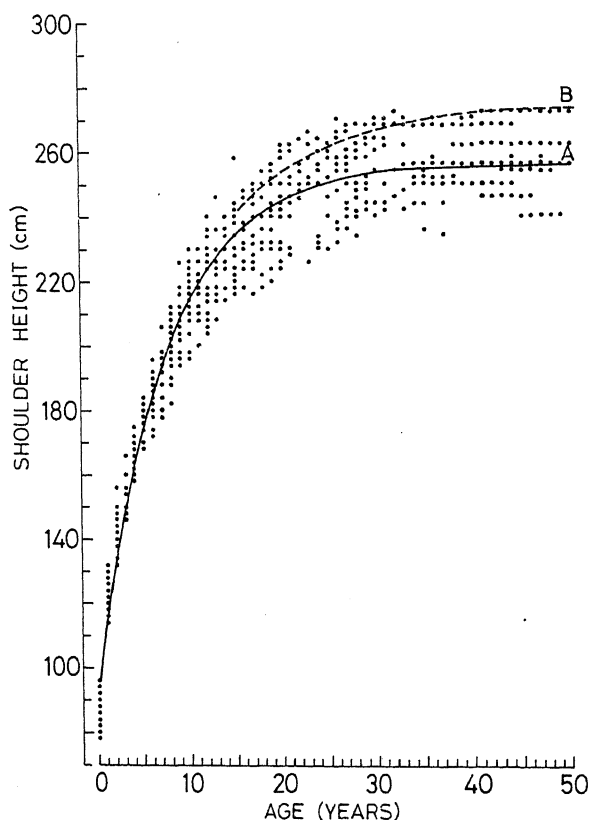
Figure 1. Growth in height in male elephants.
Plot of shoulder height (cm) against age (years). 816 measurements of 119 elephants, rounded off to the nearest 2 cm and 1 year. Data are given only up to 50 years for brevity.
A. Fitted von Bertalanffy equation (0–60 years) is $H_t = 256\{1 - \exp[-0.133(t + 3.58)]\}$ cm.
B. Presumed growth in wild bulls drawn by eye taking the higher asymptote of 274 cm (details in text).

measurements, 27 elephants), which gave a good fit for this age range

$$H_t = 232 \{1 - \exp[-0.266(t - 6.13)]\} \text{ cm.}$$

A third equation was derived for cows combining the data for 0–70 years (696 measurements, 93 elephants), which gave a good fit only above 3 years (figure 2)

$$H_t = 232 \{1 - \exp[-0.140(t + 3.85)]\}$$

3.1.3 *Check on accuracy of ageing captured elephants*: To check the extent to which any slight inaccuracies in ageing captured elephants would influence the results, the following check was made. The parameters of the von Bertalanffy equation were derived for two modified sets of data, one in which the age at capture was uniformly increased by 2 years and the other in which it was lowered by 2 years for all elephants. The resulting values of the parameters could be hardly distinguished from the earlier values. Since a systematic error of over 2 years in ageing young captured elephants is highly unlikely, we conclude that the procedure adopted in ageing captive elephants was robust and hence acceptable.
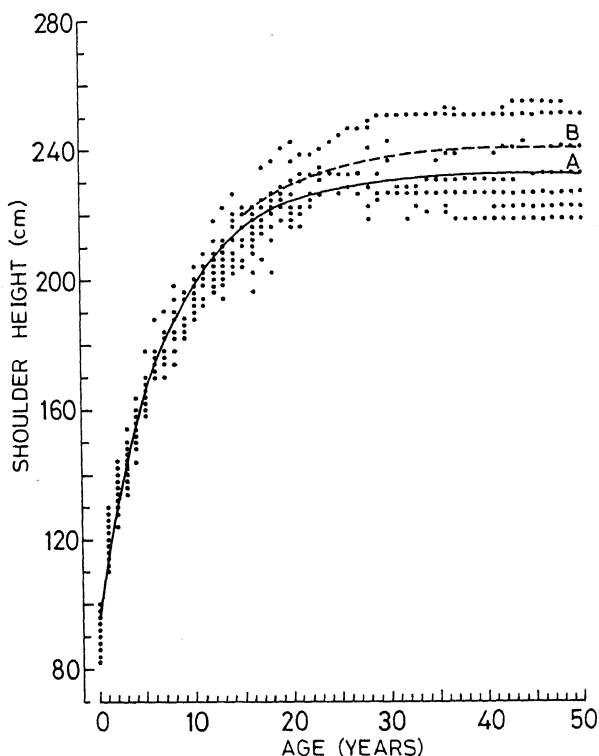
Figure 2. Growth in height in female elephants.
Plot of shoulder height (cm) against age (years). 696 measurements of 93 elephants, rounded off to the nearest 2 cm and 1 year. Data are given only up to 50 years for brevity.
A. Fitted von Bertalanffy equation (0–60 years) is $H_t = 232 \{1 - \exp[-0.140(t + 3.85)]\}$ cm.
B. Presumed growth in wild cows drawn by eye taking the higher asymptote of 240 cm (details in text).

3.1.4 *Differences in growth rates of captive and wild elephants*:   If these growth equations were to be used for ageing wild elephants it had to be confirmed that captive and wild elephants did not differ in their rates of growth. There were, in fact, reasons to believe that elephants in captivity were stunted in height compared to wild elephants. We carried out the following test to resolve this issue. The asymptotic heights of both males and female elephants were considered under these categories:

(A) Elephants born in captivity and those captured below age 10 years.
(B) Elephants which were above age 15 years when captured and wild adult elephants whose heights were estimated.

For captive elephants the criterion for determining asymptotic height was that they should have shown no increment in height for at least 4 successive years. Only such elephants were included in the analysis. The mean asymptotic heights grouped into two categories for male elephants are given in table 1.

An interesting observation is that the mean asymptotic height in category (A) is the same as the computer-fitted asymptotic height for bulls aged 15–60 years. This is not surprising since only bulls born in captivity or captured below age 8 years were used in fitting the equation.

The two means are seen to be significantly different ($t = 4.189$, $df = 42$, $P < 0.001$). It can thus be concluded that bulls which spend their pre-pubertal years in captivity attain a lower maximum height on the average compared to bulls in the wild or those captured during adult stage. It is possible that the secondary growth spurt in such captive elephants is reduced due to excessive work (in logging and other activities) and insufficient nutrition.

The fitted equation using data only on captive elephants cannot be directly used to estimate the age class of wild elephants especially after 15 years. The data from category (B) suggest that wild bulls may attain an asymptotic height of 274 cm on the average. From 15 years onwards another curve was drawn by eye which could represent the growth of wild bulls (figure 1).

A similar exercise was carried out for female elephants (table 2). The two means are significantly different ($t = 4.36$, $df = 86$, $P < 0.001$). Female elephants growing in captivity also attain a lower mean asymptotic height than those in the wild. The mean height in category (A) is the same as the H_∞ value derived from the computer fit. For use in describing growth in wild female elephants a curve has been drawn by eye taking the higher asymptotic value in category (B) of 240 cm (figure 2).

Table 1. Mean asymptotic heights of male elephants.

	Born in captivity or captured below age 10 years (A)	Captured after age 15 years or photographed in the wild (B)
Mean asymptotic height (cm)	258·2	273·7
Standard deviation	10·60	13·95
Sample size (n)	26	18

Table 2. Mean asymptotic heights of female elephants.

	Born in captivity or captured below age 10 years (A)	Captured after age 15 years or photographed in the wild (B)
Mean asymptotic height (cm)	231·4	239·5
Standard deviation	9·83	6·40
Sample size (n)	20	68

3.2 *Growth in body weight*

The computer-fitted parameters of the von Bertalanffy equation for growth in body weight with age in captive elephants are as follows.

3.2.1 *Male elephants*: (130 measurements of 22 elephants in the age range 0–60 years)

$$W_t = 3255 \{1 - \exp[-0 \cdot 149\,(t + 3 \cdot 16)]\}^3 \text{ kg}.$$

This gave a good fit to the data for age 2–60 years. The low asymptotic weight of 3255 kg refers only to captive elephants. From the height-body weight relationship (see below) it was seen that this weight corresponds to a bull of 258 cm height. Bulls in the wild would attain a much higher weight since they also attain a higher mean maximum height (274 cm). The predicted weight for a 274 cm tall bull is about 4000 kg. A curve was drawn by eye to depict the growth in weight of wild bulls above 15 years with this higher asymptotic value (figure 3).

3.2.2 *Female elephants*: (100 measurements of 29 elephants in the age range 0–70 years)

$$W_t = 3055 \{1 - \exp[-0 \cdot 092\,(t + 6 \cdot 15)]\}^3 \text{ kg}.$$

This gave a good fit to the data for 2–70 years (figure 4). Weights of both pregnant and non-pregnant females have been combined for this fitting. From the height-body weight relationship this asymptotic weight corresponds to a female of 247 cm height, which is higher than the asymptotic height (240 cm) attained by these in the wild. The curve for body weight with age may have been influenced towards a higher asymptotic value because the sample of elephants used for deriving the equation contained a number of females captured after age 15 years (which were omitted for the growth in height equation). Also, one cow weighing 3750 kg was exceptionally heavy. Body weight would also fluctuate to a certain extent (unlike height) depending on the season, on whether the cow is pregnant or not and so on. Considering all aspects it can be concluded that female elephants in captivity do not show any reduced growth in body weight.

3.3 *Tusk growth in bulls*

The length of exposed tusks in a male elephant may be a poor indicator of age partly due to the high variation in degree of wear, but the circumference of the tusk
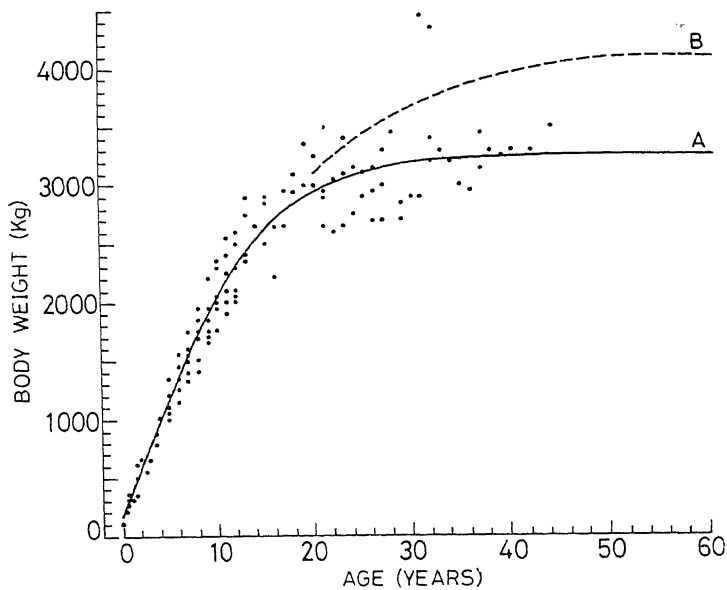
Figure 3. Growth in body weight in male elephants.
Plot of body weight (kg) against age (years). 130 measurements of 22 elephants, rounded off to the nearest 50 kg (for those above age 2) and 1 year.
A. Fitted von Bertalanffy equation is $W_t = 3255 \{1 - \exp[-0.149(t + 3.16)]\}^3$ kg.
B. Presumed growth in wild bulls drawn by eye taking a higher asymptote of 4000 kg.
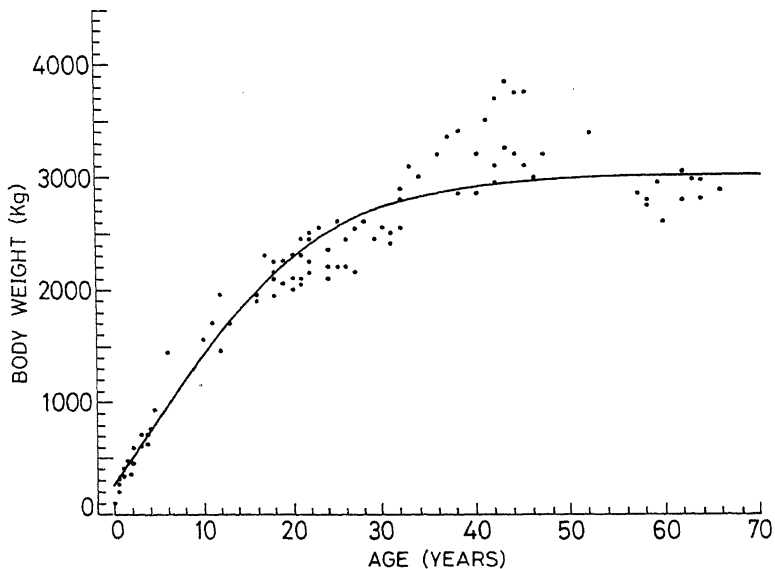
Figure 4. Growth in body weight in female elephants.
Plot of body weight (kg) against age (years). 100 measurements of 29 elephants, rounded off to the nearest 50 kg (for those above age 2) and 1 year.
Fitted von Bertalanffy equation is
$$W_t = 3055 \{1 - \exp[-0.092(t + 6.15)]\}^3 \text{ kg.}$$

at lip line (CTLL) is not affected (Hanks 1972). We examined the records (and some tusks) of 247 pairs of tusks obtained from wild bulls and also measured tusk size in 30 captive bulls which could be aged reasonably accurately. Although the sample sizes are small it was seen that the maximum CTLL (below 40 cm) in captive bulls was much lower than that attained in the wild (48 cm). After fitting von Bertalanffy equations for various age ranges it was found that the equation for age 2–25 years gave a good fit for captive bulls in this age range and could also be extrapolated for wild bulls above this age. The parameter t_0 was set to zero since no measurable tusks are seen at birth. The derived equation for CTLL with age is

$$CTLL_t = 43 \cdot 4 \left\{ 1 - \exp\left[-0 \cdot 064 \, t \right] \right\} \text{ cm.}$$

From 211 pairs of tusks the CTLL and the weight were obtained. Since there is a distinct tendency in elephants to wear out one tusk faster than another, we took the weight of only the intact tusk in each pair. The cube-root of tusk weight when plotted against CTLL gave a linear relationship and a regression was fitted

$$\sqrt[3]{\text{tusk weight}} = (0 \cdot 0715 \times CTLL) - 0 \cdot 0888 \text{ kg}$$

$$\text{i.e. tusk weight} = \left\{ (0 \cdot 0715 \times CTLL) - 0 \cdot 0888 \right\}^3 \text{ kg.}$$

Using the tusk circumference–tusk weight relationship and the von Bertalanffy equation for growth in tusk circumference with age, a curve was drawn to describe the growth in tusk weight with age (figure 5). The result was a sigmoid growth in weight curve, which is exponential during 0–10 years, linear during 10–30 years and later shows a declining rate. In contrast, Laws (1966) described an exponential growth rate in tusk weight throughout the life-span of male African elephants. He has not given any basis for how an exponential increase in tusk weight could be sustained by the metabolism of an elephant during old age. It is more logical to believe that growth rate of tusks would decrease as of any other organ due to increased catabolism.

3.4 Height-weight relationship

Hanks (1972) showed that in African elephants above 30 years of age the height increases much less than weight (allometric growth). He suggested that the relationship between these two body measurements would be best expressed by a semilog plot, log body weight on shoulder height. We found that a linear regression of cube root of body weight on shoulder height gave a better fit than the semilog plot. The height-weight relationship derived for both male and female elephants were very similar.

Male elephants (130 height-weight measurements from 22 elephants)

$$\sqrt[3]{\text{weight}} = (0 \cdot 057 \text{ height in cm}) + 0 \cdot 114 \text{ kg}$$

$$\text{i.e. weight} = \left\{ (0 \cdot 057 \text{ height in cm}) + 0 \cdot 114 \right\}^3 \text{ kg.}$$

Female elephants (100 height-weight measurements from 29 elephants)

$$\sqrt[3]{\text{weight}} = (0 \cdot 060 \text{ height in cm}) - 0 \cdot 335 \text{ kg}$$

$$\text{i.e. weight} = \left\{ (0 \cdot 060 \text{ height in cm}) - 0 \cdot 335 \right\}^3 \text{ kg.}$$
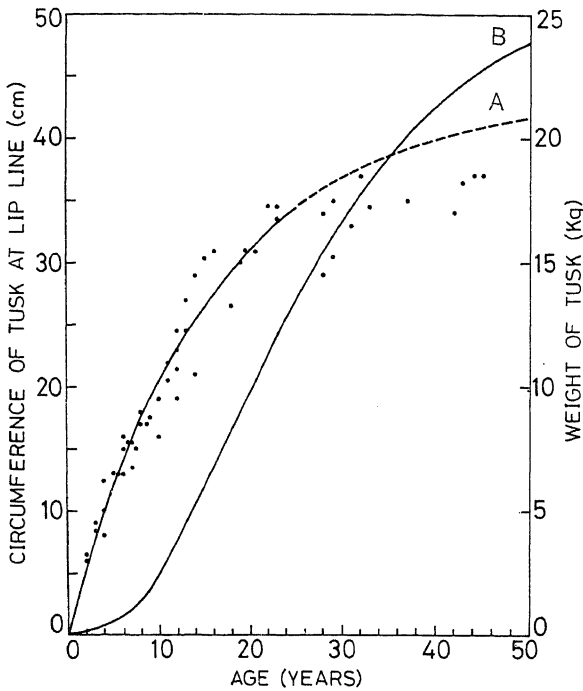
Figure 5. Tusk growth in male elephants.
Plot of circumference of tusk at lip line (CTLL in cm) against age.
A. Fitted von Bertalanffy equation for growth in CTLL using data for 2–25 years.
$CTLL_t = 43.4 \{1 - \exp[-0.064t]\}$ cm.
B. Growth in tusk weight (kg), based on the relationship between CTLL and tusk weight (see text for details).

3.5 Height-circumference of front foot relationship

It has been the standard practice in Asia to take the height of an elephant as twice the circumference of front foot (CFF) measured at the sole. To see whether this relationship holds good over the entire lifespan of the animal, the height/CFF ratios were plotted against the CFF and a linear regression fitted. There was practically no difference between male and female elephants and therefore the equation for the combined data is given below (353 height-CFF measurements from 111 elephants).

$$\frac{\text{Height}}{\text{CFF}} = 2.03 - (0.0004) \text{ CFF}.$$

This is the same as saying that

$$\text{Height} = 2.03 \text{ CFF}.$$

For practical purposes twice the CFF can be taken as the height, which confirms the traditional estimate although the range of height/CFF ratio went from 1.74

2·18. The slope of the regression is also practically zero, strongly suggesting that the relationship is isometric, that is, the shoulder height and CFF increase at the same rate throughout the lifespan of the animal. The CFF provides another criterion by which to estimate the age of an elephant.

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SUBJECT INDEX

AUTHOR INDEX

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The paper must be divided into sections starting preferably with 'Introduction' and ending with 'Conclusions'. The main sections should be numbered 1, 2, etc., the sub-sections 1.1, 1.2, etc., and further sub-sections (if necessary) 1.1a, 1.1b, etc. All measurements should be given in SI units (see *Nature London* **216** 1272–1274, 1967). Arabic numerals should be used, for time, dimensions, distances and weights (e.g. 2h, 2.5 μ (mu), 3 cm, 5 g), otherwise numbers of less than three figures should be spelt out, except where numbers occur frequently in a sentence or paragraph or in a group. Avoid figures at the beginning of a sentence. Give dates in the form: 11 August 1975. The scientific names of genera and species are printed in italics and therefore should be underlined in the typescript. Papers relating to descriptions of taxa should be based on a reasonably good series of specimens and not on odd material. Authors of names of taxa should be cited in the summary and at the first mention of a taxon in the text, but not elsewhere. Accepted common names of plants and animals (and other organisms) and of plant and animal or other diseases should neither be capitalised nor placed within quotation marks. Words and phrases of foreign origin but not in common use (e.g., in vitro, in situ) are printed in roman type. The attention of authors proposing new taxa is drawn to the need to give a Latin diagnosis, adequate descriptions and figures, and to designate the types as required by the International Code of Nomenclature. The description of a new species should be accompanied by a figure and the type specimen wherever possible should be deposited in one of the National herbaria or other collections.

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